The Journal

of

The Indian Botanical Society

(Formerly "The Journal of Indian Botany")

EDITED BY

M. O. P. IYENGAR

Vol. XXIV
1945

PRINTED AT THE BANGALORE PRESS, MYSORE ROAD
BANGALORE CITY

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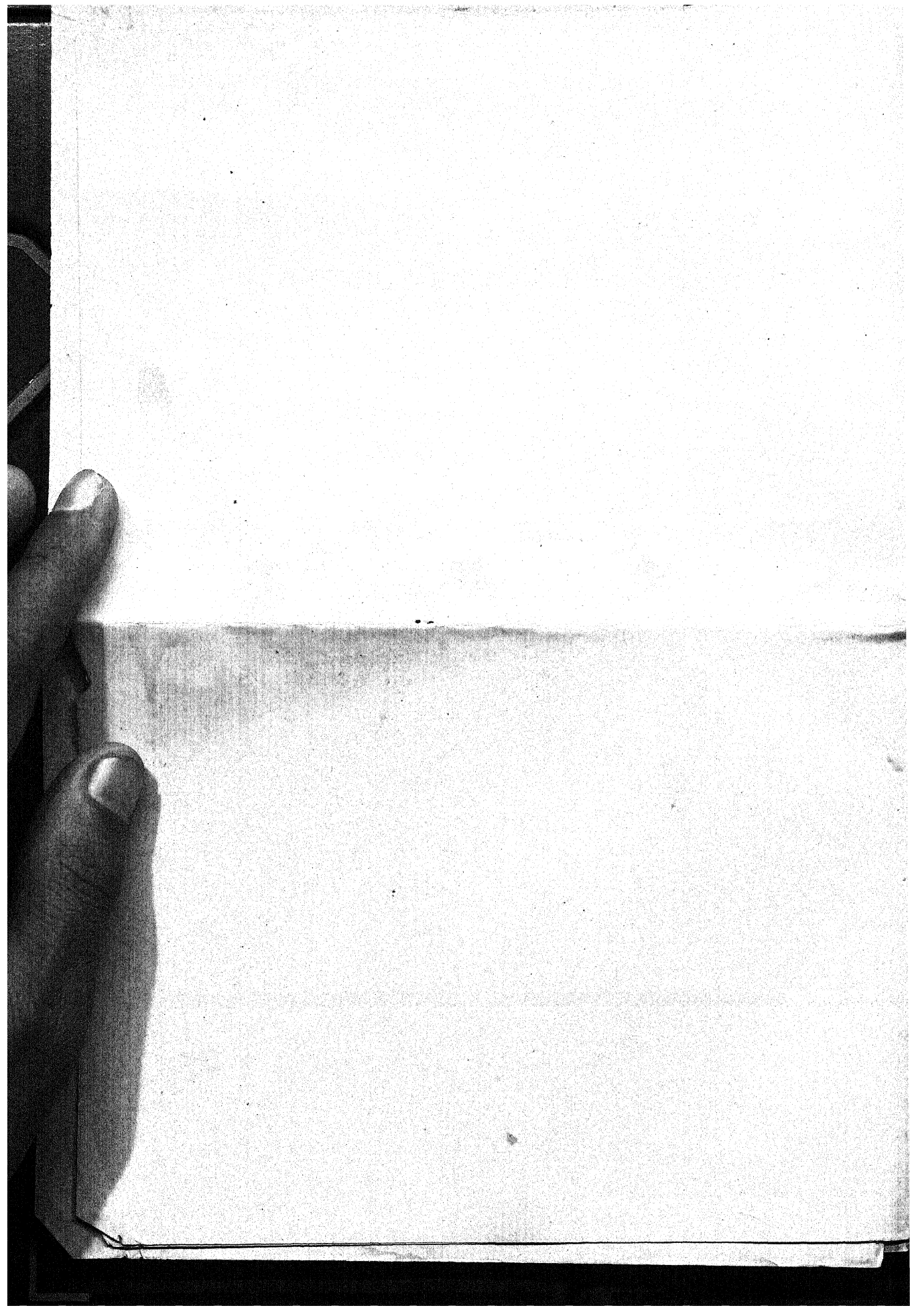


The Journal of the Indian Botanical Society

(Formerly "The Journal of Indian Botany")

VOL. XXIV] FEBRUARY, 1945 [No. 1

THE EMBRYO-SAC OF *HECKERIA SUBPELTATA* KUNTH.

BY B. G. L. SWAMY

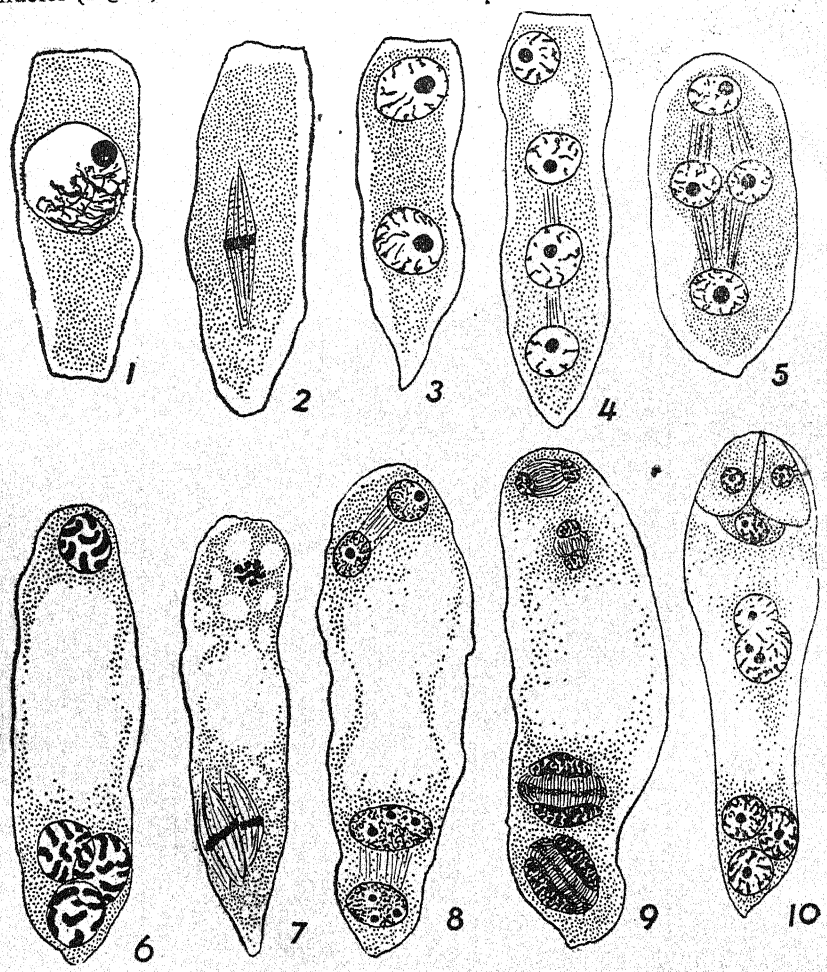
Received for publication on November 16, 1944

Heckeria umbellata and *H. peltata* were first investigated by Johnson (1902), who showed that the development of the embryo-sac in these species followed the *Adoxa*-type. Schnarf (1931, 1936) and Maheshwari (1937) made a careful study of Johnson's figures and opined that the *Fritillaria*-form would be the actual course of development of the female gametophyte. This surmise is borne out by a reinvestigation of *Heckeria umbellata* by Maheshwari and Gangulee (1942). *H. subpeltata* Kunth. is an uninvestigated species which grows in a wild condition in the evergreen forests of the Western Ghats. The results of a study of the development of the female gametophyte of this plant are presented in this paper.

OBSERVATIONS

The ovary and ovule present the same topographical and structural features as in *Heckeria umbellata* (Maheshwari and Gangulee, 1942). The archesporial cell is hypodermal in origin and cuts off a parietal cell, which divides only once in the majority of instances. The megaspore mother-cell enlarges and elongates lengthwise (Fig. 1). Its nucleus undergoes the characteristic pre-meiotic changes and divides into two nuclei (Figs. 2 and 3), which in turn complete the meiotic divisions by forming four megaspore nuclei which are not separated by walls; the arrangement of the megaspore nuclei may be linear (Fig. 4) or more or less quadripolar (Fig. 5). At about this stage, one can notice a small vacuole appearing between the micropylar and the remaining three megaspore nuclei (Fig. 4). Finally the vacuole enlarges to such an extent so as to push away the micropylar nucleus and the three nuclei to opposite poles (Fig. 6). In this condition the nuclei begin to divide and the spindles of the three chalazally situated nuclei come still closer so that their equatorial regions lie more or less in a single line and plane (Fig. 7); but at the onset of anaphase, the

individuality of the three spindles is lost and they merge into a single large division figure, which produces two large triploid nuclei (Fig. 8). This stage of the embryo-sac, which shows two haploid nuclei at the micropylar and two triploid nuclei at the chalazal end, is the "Secondary four-nucleate stage". One more division of all the four nuclei (Fig. 9) results in an "8-nucleates" embryo-sac (Fig. 10), which,



Figs. 1–10.—Fig. 1. Megaspore mother-cell. Fig. 2. Division of the megaspore mother-cell. Fig. 3. 2-nucleate embryo-sac. Fig. 4. 4-nucleate embryo-sac in which the nuclei are arranged in a linear row. Note the vacuole between the micropylar nucleus and the rest. Fig. 5. 4-nucleate embryo-sac in which the nuclei are disposed in a quadripolar manner. Fig. 6. 1 plus 3 arrangement of the four nuclei. Fig. 7. Division of the "primary four nuclei" into the "secondary four-nucleate" embryo-sac; note the extremely juxtaposed spindles at the chalaza. Fig. 8. "Secondary four-nucleate embryo-sac." Fig. 9. The last (fourth) division of the embryo-sac nuclei. Fig. 10. Mature embryo-sac. All Figures × 900.

however, is equivalent to a tetrasporic 16-nucleate condition (*cf.* Swamy, 1944). The egg apparatus is organised by the haploid nuclei and the secondary embryo-sac nucleus is formed by the fusion of the haploid upper polar nucleus and the triploid lower polar nucleus.

SUMMARY

The female gametophyte of *Heckeria subpeltata* Kunth. has been studied and its development is shown to correspond to the *Fritillaria*-form.

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A NOTE ON THE LIFE-HISTORY AND THE SYSTEMATIC POSITION OF *RHINOSPORIDIUM SEEBERI* (WERNICKE)

BY H. CHAUDHURI, BALBIR SINGH AND KARTAR SINGH THIND

Botany Department, Panjab University, Lahore

Received for publication on September 20, 1944

VARIOUS cases of rhinosporidiosis on man, horse and cow have been reported from all over the world. In India it has been observed in Bengal, Madras, Poona and other parts (Allen, 1935; Allen and Dave, 1936; Anantnarayan Rao, 1938; Beattie, 1906; Cherian and Vasu Devan, 1929; Karunaratne, 1936; Krishna Murti, 1931; Kurup, 1931; Mandlik, 1937; Noronha, 1933; Norrie, 1929; Sahai, 1938).

In the present case the rhinosporidiosis has been studied on cow, bullock and pony. The material was obtained by one of the authors (Balbir Singh) from various places in C.P. The nasal polypi of these animals along with their fæces and nasal excretions were fully studied. The microtomic sections of the polypi were also prepared.

The systematic position of the causal organism, *Rhinosporidium seeberi* has been so far a disputed question. An attempt has been made in the present publication to throw some light on this.

Young Stage

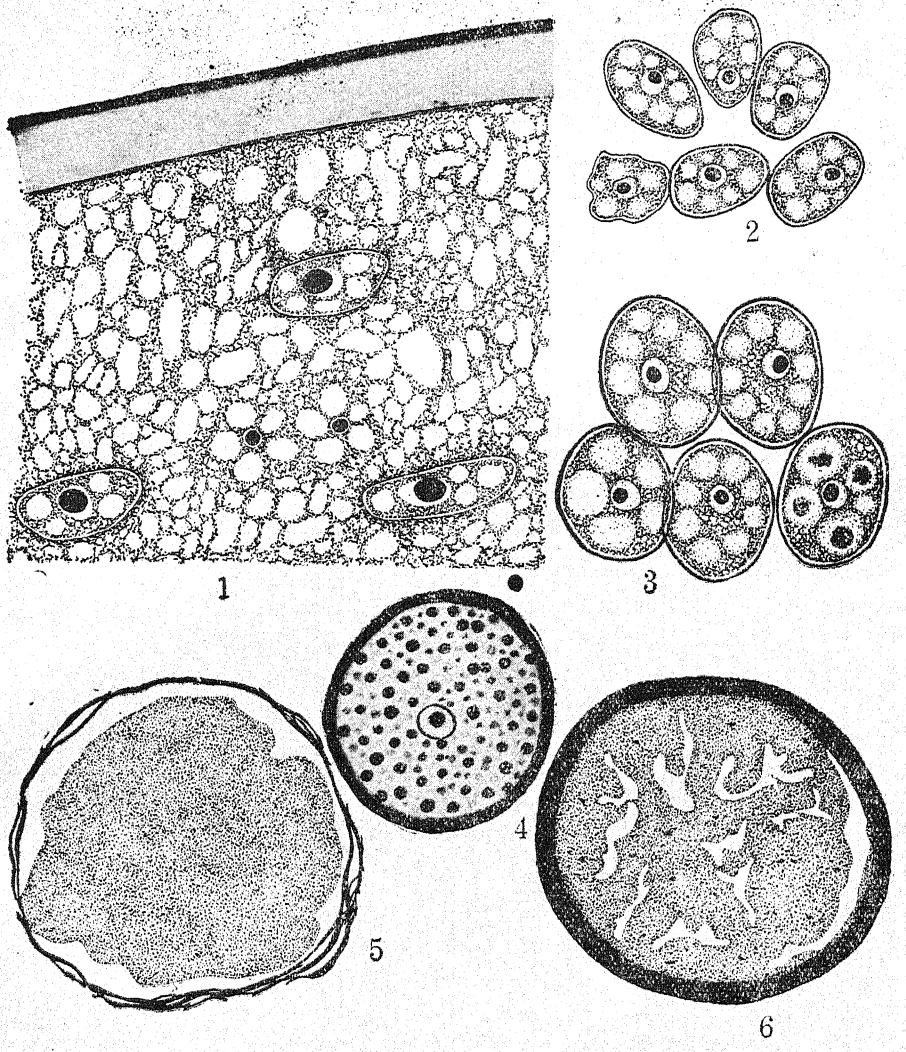
The parasite, as far as observed, starts its life-history with a small, spherical, oval or oblong body, sometimes with irregular boundary, inside the connective tissue cells of the polypus (Fig. 1). It measures 5–9 μ, the average being 6–8 μ in diameter. There is a nucleus with a distinct karyosome. The cytoplasm is granular and contains a few spherules (Figs. 2 and 3).

Trophic Stage

The parasite then enters a period of very active growth and considerably enlarges in size with corresponding accumulation of nutritive material in the form of spherical globules and increase in the size of the nucleus and the thickness of the wall (Figs. 4 and 12).

During the earlier part of the trophic stage the parasite remains more or less roundish and measures 13 μ to 65 μ in diameter. The wall is 1·3 μ to 5 μ in thickness and the size of the nucleus is 4 μ in a parasite which is 38 μ in diameter while it increases to 7 μ where the parasite attains the size of 60 μ in diameter. Later on the parasite becomes perfectly oval (Fig. 13) and measures from 91 μ to 130 $\mu \times 74 \mu$ to 78 μ. The thickness of the wall increases upto 9 μ as observed in a parasite

80 μ in diameter. In larger parasites the wall is comparatively thinner, being 7–8 μ thick.

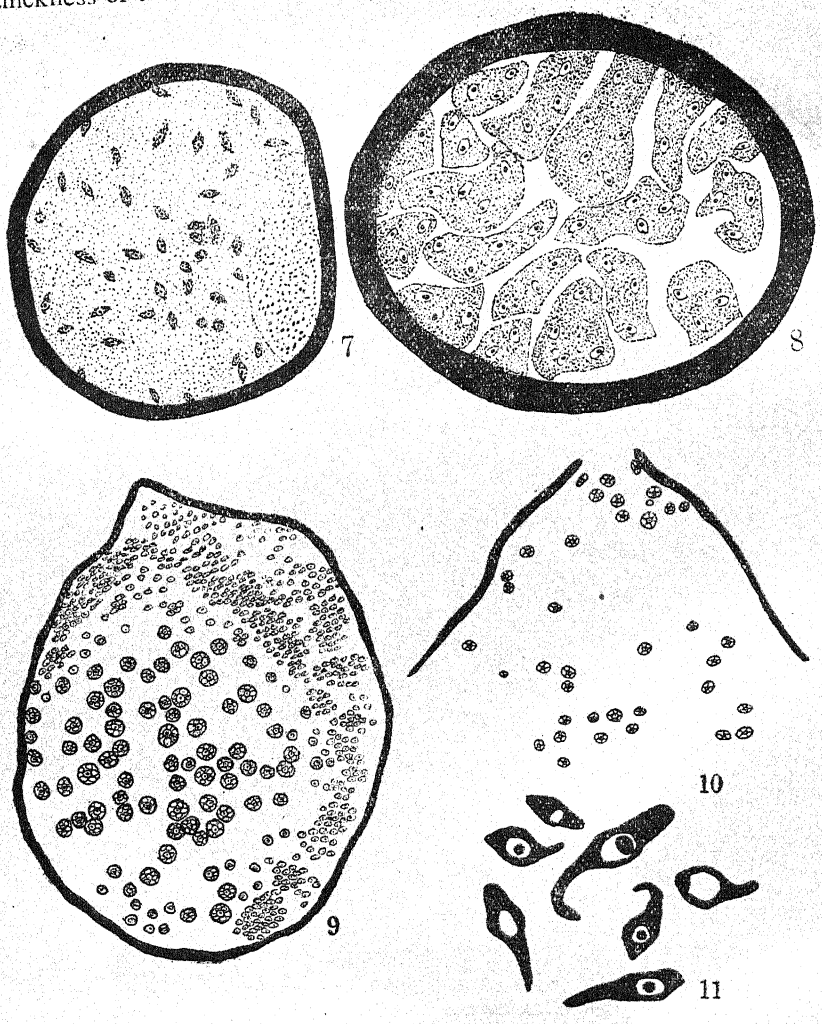


Figs. 1-6.—Fig. 1. Section of the polypus with spores in the connective tissue cells. Fig. 2. Young spores showing envelope, the nucleus with karyosome, the cytoplasm and the vacuoles. Fig. 3. Spores at a later stage of development than in Fig. 2. Fig. 4. Trophic stage. Fig. 5. Showing reduction in the size of the spherules and globules. Fig. 6. The nuclei formed after mitotic division. Figs. 1–3, × 2700; Fig. 4, × 1500; Figs. 5 and 6, × 510.

An important change now takes place in the size of granules and spherules, which were much larger in the beginning but become reduced in size later on, just prior to the nuclear division (Fig. 5).

Nuclear division

After the maximum growth period the nucleus of the parasite shows very active mitotic division. Several thousand nuclei are thus formed before the commencement of the cytoplasmic division (Figs. 6–7). The size of the parasite goes on enlarging and during the period of cytoplasmic division it varies from 144 μ to 109 μ × 130 μ to 90 μ. The thickness of the wall is from 4–9 μ.



Figs. 7–11.—Fig. 7. More nuclei formed after mitotic division. Fig. 8. Cytoplasmic division in progress. Fig. 9. Mature sporangium with beak at the top. Fig. 10. Pore differentiated at the top of the sporangium. Fig. 11. Germination of spores giving rise to amœboid structures. Figs. 7 and 8, × 510; Figs. 9 and 10, × 700; Fig. 11, × 1620.

Formation of Spores

Cytoplasmic division now sets in which is fully illustrated in Fig. 8. It goes on till there are formed uninucleate protoplasmic masses (Fig. 9). These round off and a wall is laid down around each. The parasite now represents a young sporangium with numerous uninucleate spores (Fig. 9). These young sporangia vary in size from 187 μ to 110 $\mu \times 156\,\mu$ to 110 μ while the spores measure from 3–4 μ in diameter. It is interesting to note that the central spores are differentiated earlier than the peripheral ones (Fig. 9).

The sporangia and the spores further increase in size. The fully ripe sporangia measure from 500 μ to 400 $\mu \times 400\,\mu$, while the spores reach 6–9 μ in diameter. These measurements are much higher than those given by Ashworth (1924).

Dispersal of Spores

At any point the wall of the sporangium may protrude out into a beak (Fig. 9). The beak later on breaks and a pore is formed through which the spores are discharged (Fig. 10).

Germination of Spores

Numerous spores from which blunt processes were seen in all stages of development were observed by the authors (Fig. 11). The amœboid structures thus formed seem to be the germinating spores of *Rhinosporidium seeberi* and were found in the nasal excretions. These no doubt bring about new infection.

It has, however, not been possible to carry out the artificial germination of these spores.

Systematic Position of Rhinosporidium seeberi

This organism was first seen by Seeber in 1896 in nasal polypi of man in Buenos Aires, which he described as a sporozoal parasite (Seeber, 1900) and Wernicke named this parasite as *Coccidium seeberi* in 1900. Belou (1903) in his treatise on animal parasitology described it as *Coccidium seeberi* Wernicke, 1900. Minchin and Fantham described *Rhinosporidium kinealyi* as a new genus and a new species from nasal polypi in man from India. Beattie (1906) also described *Rhinosporidium kinealyi* from Cochin material, obtained by Dr. Nair of Madras. Seeber's parasite is a *Rhinosporidium* and is the same as *R. kinealyi*. Fantham, Stephens and Theobold (1916) call it *R. kinealyi* (or *seeberi*). The question of priority of name has been discussed by Seeber (1912) and as pointed out by Hartmann (1921) the *Rhinosporidium seeberi* Wernicke has priority over *R. kinealyi*. From the nasal septum of a horse in South Africa, Zschokke (1913) described *R. equi*, a new species. That there is any specific difference between the human and equine form seems doubtful (Wenyon, 1926). All these authors regarded *Rhinosporidium* as a protozoa. Ridewood and Fantham (Fantham, 1907) in their classification put *Rhinosporidium* in subsection Polysporulea under Haplosporidia. Doflein (1906) also retained it in

Polysporulea but stated that it had many resemblances with *Chytridineæ*. Ashworth (1923) conclusively demonstrated that these were vegetable parasites. He gave a detailed account, calling it *Rhinosporidium seeberi* and related it with lower fungi for the following reasons:

(1) Presence of fatty reserves, (2) repeated nuclear division preparatory to spore formation, (3) division of cytoplasm at a later stage, (4) absence of residual cytoplasm, (5) presence of a mucoid substance between the spores, (6) wall being made up of cellulose and (7) formation of a definite pore in the sporangium.

As the thallus in *Rhinosporidium* is formed of a single cell and the mycelium is wholly lacking, Ashworth put the organism under Chytridineæ. The thallus of *Rhinosporidium seeberi* is holocarpic, *i.e.*, later on gives as a whole to the sporongium. So he put it in the family *Olpidiaceæ* of Chytridineæ.

The occurrence of germinating spores giving rise to amœboid structures as observed by the authors, indicates the affinity of *Rhinosporidium seeberi* to Chytridiales. The formation of zoospores has been suppressed here probably due to its peculiar mode of existence on man and other animals (Negroni, 1931).

Ainsworth and Bisby (1943) wonder if *Rhinosporidium* be put under *Endomycetales*. But from the evidence put forward it appears that it should be placed under *Chytridiales*.

It may be mentioned here that Anantnarayan Rao (1938), in his paper while giving a brief account of the organism, refers to both sporangia and asci. It seems that he has confused the two terms.

SUMMARY

Rhinosporidiosis occurring on cow, bullock and pony has been studied. The fæces and the nasal excretions were also examined. The amœboid structures formed from the germination of spores of *Rhinosporidium seeberi* were found in the nasal excretions. These structures further strengthen the affinity of *R. seeberi* with *Chytridiales*.

The authors have great pleasure in acknowledging their thanks to Dr. G. Watts Padwick, Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, for help with many references to literature and for kindly looking through the manuscript.

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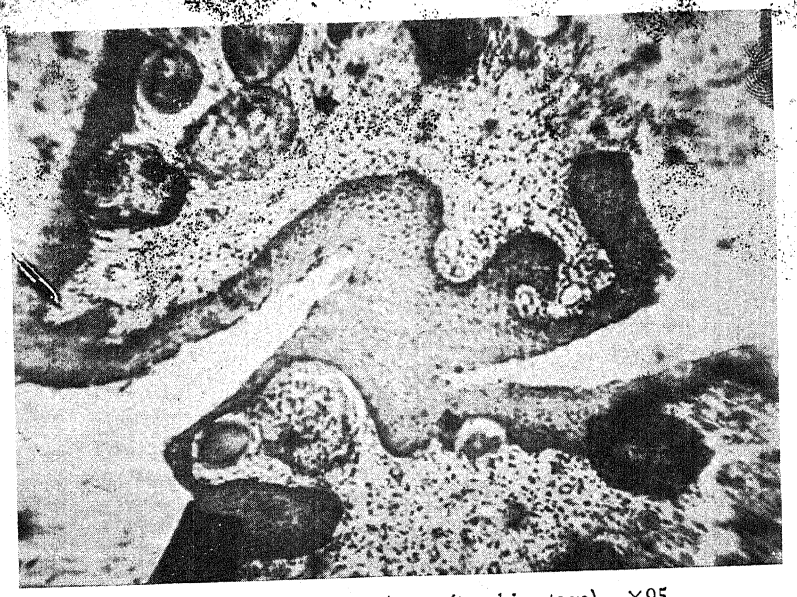


Fig. 12. Section of Polypus (trophic stage). ×95

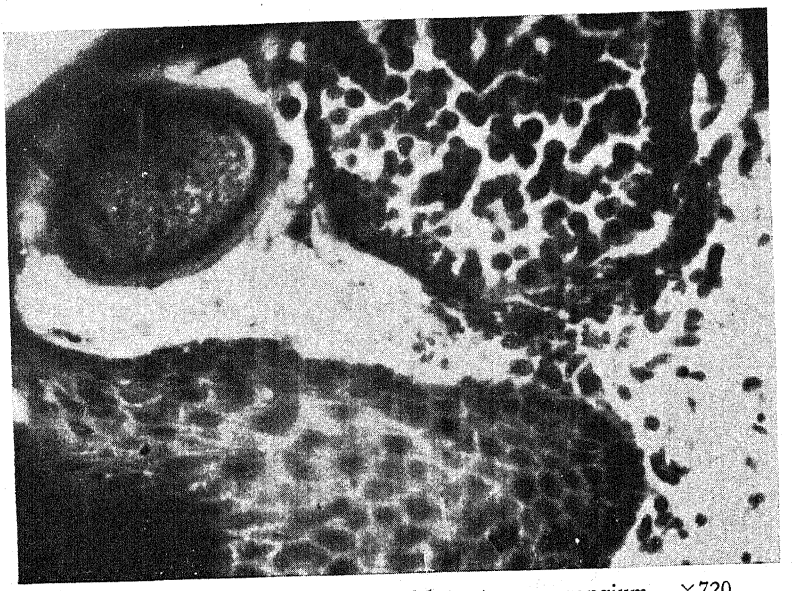
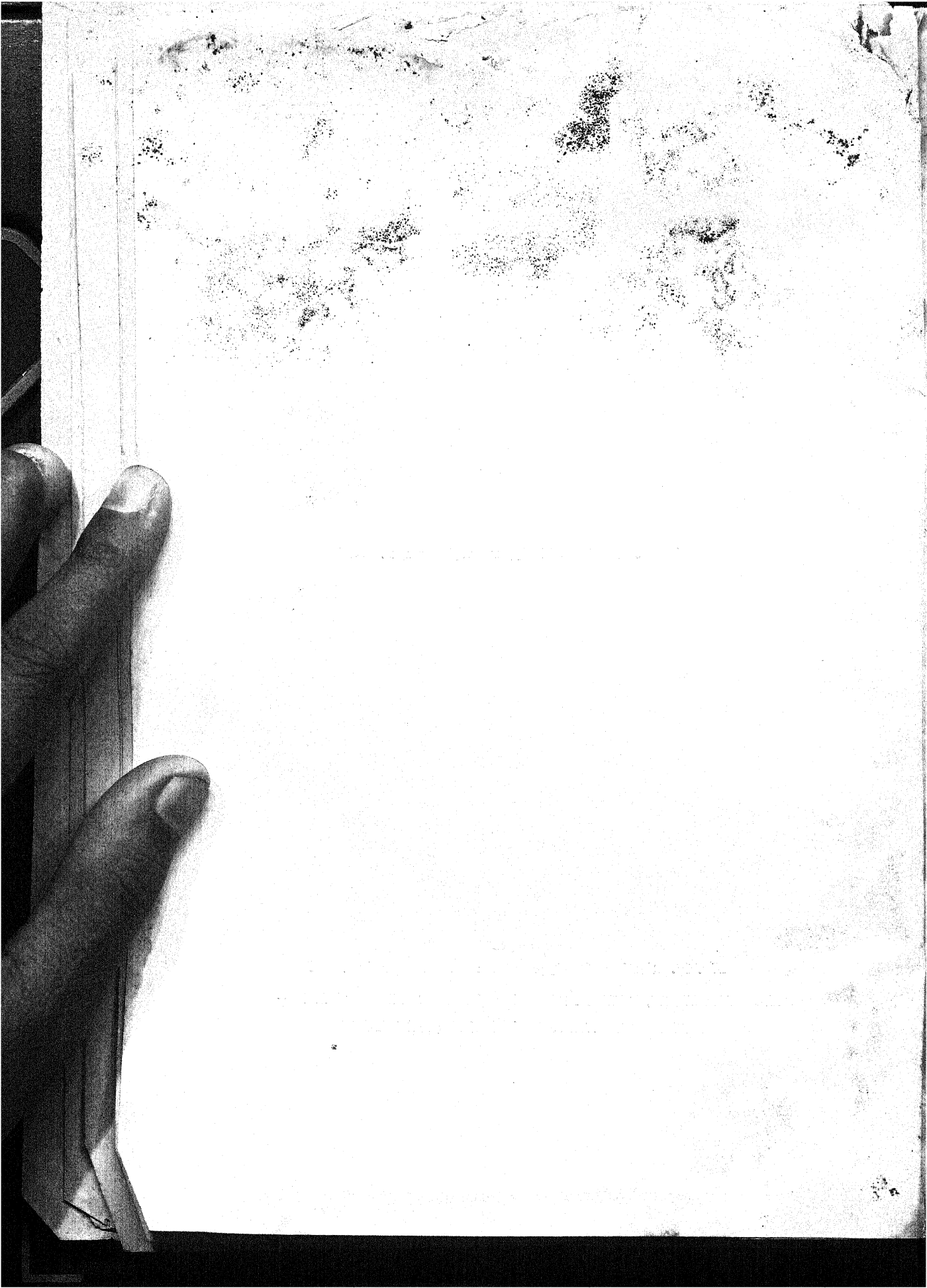


Fig. 13. Section of Polypus with mature sporangium. ×720

H. CHAUDHURI, BALBIR SINGH & KARTAR SINGH THIND—
RHINOSPORIDIUM SEEBERI (WERNICKE)



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STUDIES IN THE CÆSALPINIACEÆ

I. A Contribution to the Embryology of the Genus Cassia

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Received for publication on September 22, 1944

Our present knowledge of the embryology and cytology of the three families Mimosaceæ, Cæsalpiniaceæ and Papilionaceæ belonging to the order Leguminosæ (if we follow the classification of Hutchinson, 1926), chiefly on account of their different distribution, is very unequal. The Papilionaceæ being cosmopolitan and abundant both in Europe and N. America have received much attention, while the more tropical Mimosaceæ and Cæsalpiniaceæ have been only meagrely investigated. This fact has prompted the author to take up the study of the Cæsalpiniaceæ. The present paper deals with the structure and development of the ovule and embryo-sac of *Cassia* species. The author has started with this genus not only because it is the largest in the family and is represented by many species in this country, but also because the few observations that have been made by the earlier workers are in several cases contradictory.

Previous Work

The earliest reference to the embryology of the Cæsalpiniaceæ is found in the work of Braun (1860), who observed polyembryony in a species of *Cassia*. Later Guignard (1881) in his extensive studies on the embryology of the Leguminosæ also made some observations on the genera *Cæsalpinia*, *Cassia*, *Cercis*, *Gleditschia* and *Ceratonia*. He observed in *Cercis siliquastrum* both the chalazal megaspores often becoming 2-nucleate and each having the capacity of developing into a mature embryo-sac. Some further observations on the embryo-sac of *Cassia* were made by Hubert (1896).

Saxton (1907) worked out the structure and development of the ovule and embryo-sac of *Cassia tomentosa*. He observed a deeply situated primary archesporial cell which functions as the megaspore-mother cell without cutting off any primary wall cell. The megaspore-mother cell undergoes the two meiotic divisions in the normal manner and forms a linear tetrad of megaspores. The second megaspore from the chalazal end develops into the embryo-sac according to the *Normal* type. The mature embryo-sac at the chalazal end forms a tubular-extension which becomes filled with a row of antipodal cells, as happens in some Compositæ.

Ghose and Alagh (1933) studied *Cassia purpurea*. They found hypodermal primary archesporial cell in the ovules and the formation

of a primary wall cell. The second megaspore from the chalazal end, as in *Cassia tomentosa*, was found to develop into the embryo-sac.

Datta (1935) investigated *Cassia tora*. He found in the ovules sub-hypodermal primary archesporial cells, absence of the primary wall cells, and organisation of a linear tetrad of megaspores, out of which the chalazal one developed into the 8-nucleate embryo-sac. The antipodals, even though they were found to persist till fertilisation, remain only as free nuclei and are not organised into cells.

The latest work on the embryo-sac of the Cæsalpiniaceæ is a paper by Paul (1937) dealing with *Tamarindus indica*. He reports the differentiation of the primary archesporium from the sub-hypodermal layer, formation of the primary wall cell and a normal tetrad of megaspores from the megaspore-mother cell. The chalazal megaspore is the functional one and develops into the embryo-sac according to the *Normal*-type.

Material and Methods

During the course of the present investigation material of the following species of Cassia has been investigated.

1. *C. occidentalis* Linn.
2. *C. abtusifolia* Linn.
3. *C. glæuca* Lamk.
4. *C. glauca* Lamk. var. *suffruticosa* Koenig.
5. *C. marginata* Roxb.
6. *C. siamea* Lamk.

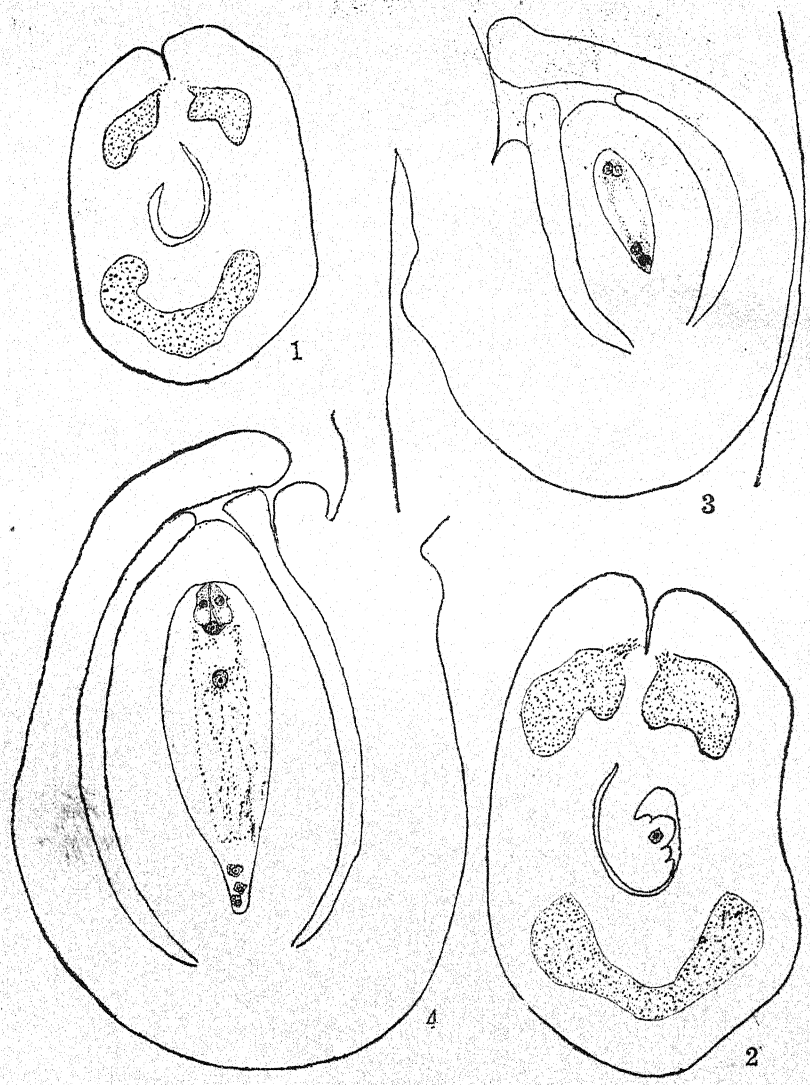
The first two species grow abundantly at Benares, particularly during the rainy season in waste places, and their material was collected from plants growing wild in the Benares Hindu University area. The material of *Cassia glauca* was obtained from a plant cultivated in the Benares Hindu University Botanical Garden, and that of *C. glauca* var. *suffruticosa* from a plant growing in the Sri Sita Ram Krishishala, Benares. The material of *C. marginata* was collected by Dr. A. C. Joshi from a tree growing in one of the gardens at Allahabad and that of *C. siamea* from trees planted on road-sides in the Benares Hindu University campus.

In all cases the material was fixed in Nawaschin's fixative between 12 noon and 3 p.m. during the months of September, October and November, 1940. An exhaust syringe was employed to cause the rapid immersion of the material in the fixative ; 12–18 hours after fixing, the material was rinsed in water four or five times and then transferred to 70% alcohol. The further dehydration and embedding in paraffin was carried out according to the customary methods. Sections were cut 8–16μ thick. Delafield's Hæmatoxylin and Newton's Iodine Gentian Violet were employed as stains.

Structure and Development of the Ovule

The ovules in all species of *Cassia* are borne in two rows along the ventral suture of the monocarpellary unilocular gynœcium, the ovules

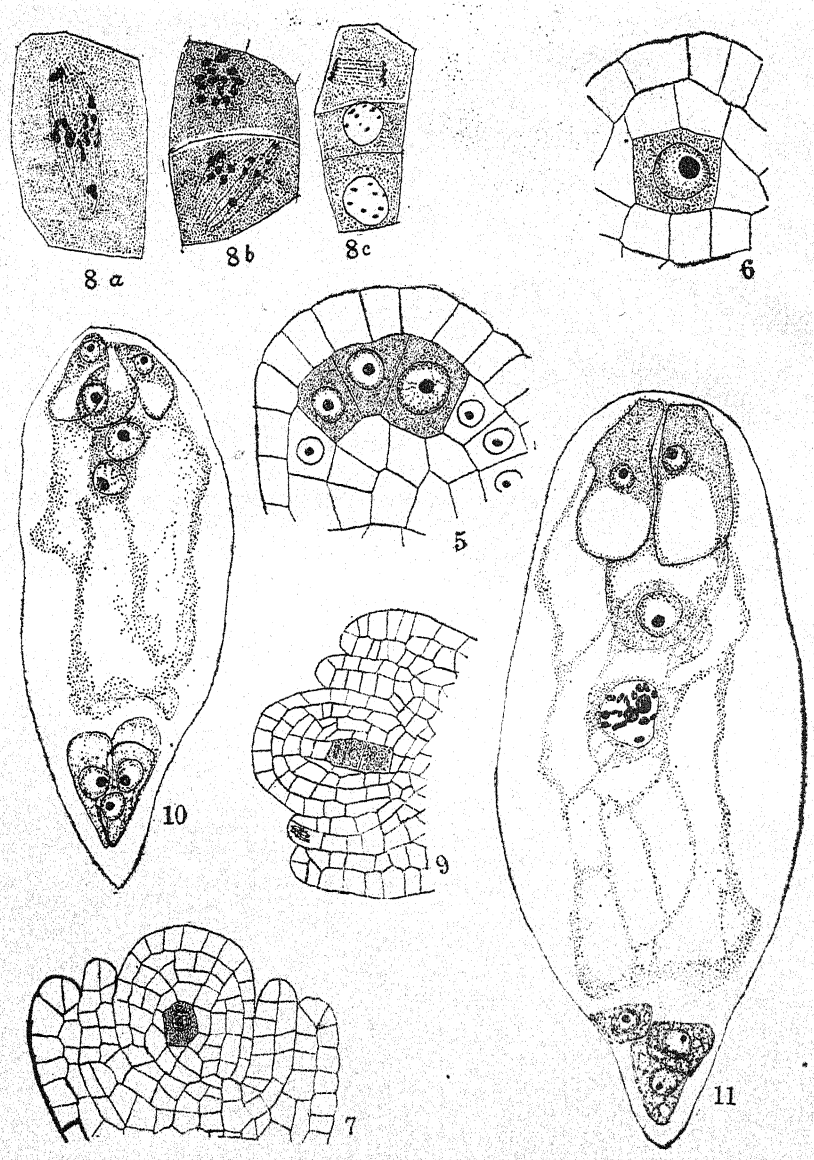
of the two rows alternating with one another. The number of ovules in a carpel varies slightly with each species, but it may be stated that on the average there are 30–50 ovules in a carpel.



Figs. 1–4. *Cassia occidentalis*.—Figs. 1 and 2. Transverse sections of young ovaries showing early stages in the development of the ovule. The vascular bundles of the carpel are stippled. Fig. 3. An ovule at the 4-nucleate embryo-sac stage. Fig. 4. An ovule at the mature embryo-sac stage. Figs. 1 and 2, × 800; Figs. 3 and 4, × 150.

The ovules first arise as small papillæ from the margins of the carpel, which has at this stage the form of a linear structure folded upwards along the midrib, a fact which agrees with the classical interpretation of carpel morphology. The two margins of the carpel are still free from each other and the carpel is open on the posterior side (Fig. 1). The development of the ovule primordia results chiefly from the activity and rapid division of groups of hypodermal cells, and after their differentiation these primordia are seen protruding into the ovary cavity (the space enclosed by the wall of the carpel). The ovule primordia at first are quite straight, but soon during further growth the cells develop more actively on one side than on the other. Consequently the young ovules bend towards the apex of the ovary, and gradually assume an anatropous form (Fig. 3). Reeves (1930) in *Medicago* observed that the curvature of the ovules is conditioned by mechanical pressure. He found as long as there is space for free development, the ovule remains orthotropous, but as soon as the ovule during its growth comes in contact with the dorsal wall of the carpel opposing it, its straight growth comes to end and it curves generally towards the base. Maheshwari (1931) in *Albizzia Lebbek* describes the young nucellus as growing at first straight and at right angles to the placenta (ventral suture), but when it approaches the dorsal wall of the carpel it begins to curve upwards. Singh and Shivapuri (1935) describe the same condition in *Neptunia oleracea*, a member of the Mimosaceæ. In a few cases, in which the carpel was found to remain open throughout its development, the ovules were found to remain permanently orthotropous. Great significance has been attached to this fact by Joshi (1935) in the evolution of the anatropous form of the ovule. In *Cassia* species studied during the course of the present investigation, however, no such relation has been found. The primordia of the ovules begin to bend towards the apex of the ovary even when these are quite away from the dorsal wall of the ovary.

The mature ovules in all *Cassia* species, even after the development of embryo, are anatropous with a slight tendency towards amphitropy (Figs. 3 and 4). They possess two integuments. The inner integument in the flowering plants generally differentiates from the ovule primordium almost simultaneously with the differentiation of the primary archesporium, but in all *Cassia* species investigated by the writer it did not appear till the primary archesporial cell had cut off the primary wall cell and had reached the megaspore-mother cell stage. The development of the integuments thus in the genus is considerably delayed. The primordium of the inner integument arises just below the level of the megaspore-mother cell (Fig. 2). Soon after its differentiation, the primordium of the outer integument appears just below that of the inner integument. In spite of the late start, the outer integument soon outgrows the inner by its faster development, so that by the time of tetrad formation the outer integument has attained a slightly greater length than the inner (Figs. 25 and 31). While the outer integument by this time has reached the level of the nucellus apex, the inner integument is seen to end somewhat below the level of the nucellus. The disparity between the growth of the two integuments is maintained



Figs. 5–11. *Cassia occidentalis*.—Various stages in the development of the embryo-sac.—Fig. 5. Apex of the nucellus showing a group of primary archesporial cells. Fig. 6. Formation of the primary wall cell and its division by an anticlinal wall. Fig. 7. An ovule showing the megaspore-mother cell. Fig. 8 *a–c*. Three stages in the development of a tetrad of megaspores; (*a*) I meiotic division in the megaspore-mother cell; (*b*) the II meiotic division; (*c*) a stage showing the formation of a T-shaped tetrad of megaspores. Fig. 9. The ovule from which the T-shaped tetrad shown in Fig. 8 *c* has been sketched. Fig. 10. A young 8-nucleate 7-celled embryo-sac. Fig. 11. A mature embryo-sac after the fusion of the polar nuclei. Figs. 7 and 9, × 800; the rest, × 1700.

even in the later stages, so that in the mature ovule (an ovule at the time of fertilisation) the micropyle is mostly formed by the outer integument. The inner integument contributes only to a very small length of the micropyle (Fig. 4). There are two further peculiarities of the micropyle. Firstly, the passage formed by the outer integument is not quite opposite to that formed by the inner. It is rather to one side, so that the micropyle is not straight but somewhat zig-zag. Secondly, at the micropyle the outer integument is never in direct contact with the inner integument. In this region there is always a small space between the two integuments. Both the integuments in all species are two layers of cells thick except near the micropyle, where both the integuments are 4–5 cells thick.

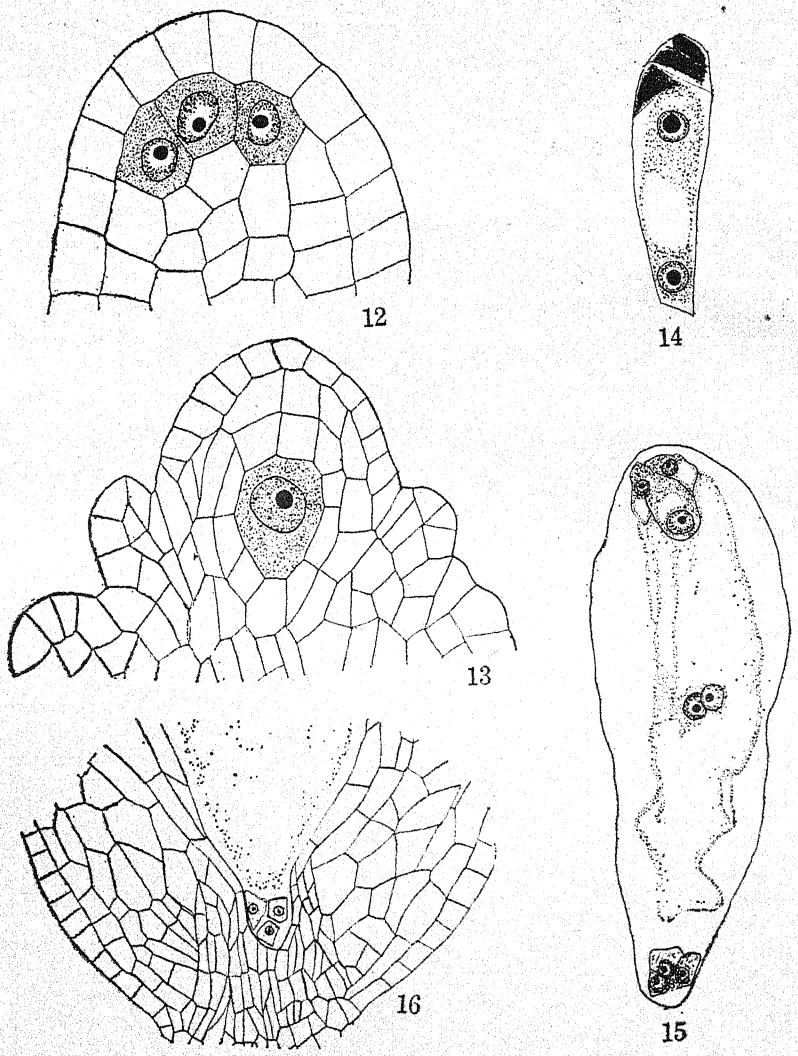
The nucellus in species of *Cassia* is massive from the very beginning. At the tetrad stage there are approximately 4–5 layers of nucellus cells above the tetrad, 3–5 layers on the sides, and 4–5 layers beneath the tetrad (Figs. 9 and 31). By the time the embryo-sac reaches the 4-nucleate stage the number of cells in the nucellus above the embryo-sac has increased to 8–10 layers due to divisions in the parietal cells. Before fertilisation many of these parietal cells are gradually crushed by the growing embryo-sac, but the number of cell layers above the micropylar end of the embryo-sac remains the same due to periclinal divisions in the epidermal cells of the nucellus. As the parietal cells are crushed at this end, the epidermal cells divide to restore the number of layers destroyed. This growth gives rise to considerable pressure inside the ovule, so that the epidermal cap shortly before fertilisation begins to project as a small beak into the micropyle of the ovule (Fig. 4). This pushes outwards the inner integument and leads to considerable decrease in the size of the air-space found between the two integuments close to the micropyle of the ovule. The formation just before fertilisation of an epidermal cap at the micropylar end of the nucellus with a small beak projecting into the micropyle seems to be a characteristic feature of all the Cassias examined by the author. I have seen it also in a number of other Cæsalpiniaceæ and perhaps this feature is characteristic of the whole family. There are approximately 6–7 layers of cells below the chalazal end of the embryo-sac, and 7–8 on the sides of the embryo-sac at the time of fertilisation. To a large extent these cell layers are soon crushed by the post-fertilisation growth of the embryo-sac.

Another characteristic feature of the ovule of Cassias is that the epidermis of the funicle and the adjacent part of the outer integument on the outer side (*i.e.*, the side on which the ovule does not bend) remains meristematic for a long time. At the tetrad stage these cells are quite distinct from the other cells of the ovule, possessing as they do dense cytoplasm and no conspicuous vacuoles. Further close to the hilum these cells grow out into a short hump-like outgrowth, which persists throughout the life of the ovule.

Development of the Embbyo-Sac

As the ovule begins to curve, but before the appearance of the integument primordia, the primary archesporium differentiates from

the other cells of the nucellus. In the flowering plants in general the curving of the ovule, the appearance of the integument initials and development of the primary archesporium are almost synchronous.



Figs. 12–16. [*Cassia obtusifolia*.—Fig. 12. Nucellus showing a group of archesporial cells. Fig. 13. An ovule at the megaspore-mother cell stage. Fig. 14. A 2-nucleate embryo-sac, with three degenerating micropylar megaspores. Fig. 15. An 8-nucleate, 7-celled embryo-sac. Fig. 16. Antipodal region of an embryo-sac showing the tubular extension of its chalazal end. Fig. 15, × 1400; the rest, × 1700.

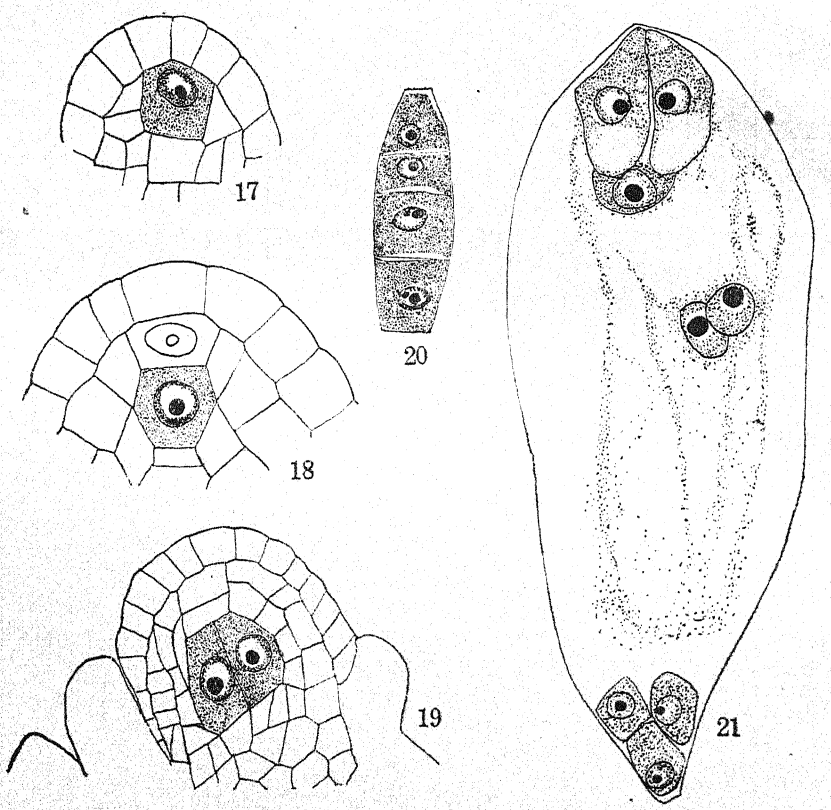
In all Cassias, however, as has been stated before, the integuments begin to develop rather late, only after the differentiation of the megaspore-mother cell in the ovules. The primary archesporium in all species

was found to be of hypodermal origin. In *Cassia glauca* and *C. glauca* var. *suffruticosa* a single primary archesporial cell is quite prominent from an early stage and can be easily distinguished from the surrounding cells (Figs. 17 and 22). In other species all the cells of the hypodermal layer are equally prominent and look just alike. They all show dense cytoplasm and possess almost equally large nuclei (Figs. 5, 12 and 26). One of these cells, however, generally the most centrally situated one, divides by a periclinal wall into an outer parietal cell and an inner megaspore-mother cell. This cell may be said to act as the primary archesporial cell (Figs. 6, 13, 18, 23, 29 and 30).

Describing the primary archesporium of the ovule, Coulter and Chamberlain (1903) state : "The archesporium is recognized by the increasing size and different reaction to stains of one or more hypodermal cells. Doubtless all of the hypodermal cells are potentially archesporial, and there is reason for believing that the deeper cells of the nucellus, most of which are probably derivatives from the original hypodermal layer, may be also. In the vast majority of the cases, however, only cells of the hypodermal layer show those changes that are characteristic of archesporial cells. It is not always easy to determine just how many hypodermal cells are to be included in the archesporium, for there is often complete gradation from cells with the size and staining reaction of undoubted archesporial cells to those showing neither increase in size nor the characteristic staining reaction. This is to be expected in case all the hypodermal cells are potentially archesporial, and there is no definite point in its history when such a cell ceases to be merely hypodermal and becomes clearly archesporial." While examining the ovules of the different species of *Cassia* for the primary archesporial stages, I have felt exactly like Coulter and Chamberlain. In the beginning in most species all the hypodermal cells at the apex of the nucellus are just similar. Then one of them cuts off a parietal cell and may be said to function as stated above as the primary archesporial cell.

Saxton (1907) noted in *Cassia tomentosa* that the primary archesporial cell is deep-seated, *i.e.*, sub-hypodermal and functions directly as the megaspore-mother cell without cutting off any parietal cell. Datta (1935) has described the same feature in *Cassia tora*. From the uniform hypodermal origin of the primary archesporium that I have noticed in the species examined by me, I am led to believe that the observations of both these authors are probably incorrect. This error has been made by them very likely from an examination of too old material, in which the parietal tissue had already begun to develop. I have not been able to see the paper by Saxton, but from examining the figures of Datta I find the Fig. 3 of his, which is almost at the same stage as Fig. 2 (and the latter is supposed to represent the primary archesporium). In the material examined by me the ovule has always developed up to the megaspore-mother cell stage by the time the integument primordia differentiate. Further, *Cassia obtusifolia* examined by me is very closely related to *C. tora*. In *Cassia obtusifolia*, I have clearly seen the hypodermal origin of the primary archesporium and the formation of a primary parietal

cell. It is not possible to believe that two such closely related species can show such a great difference in their embryological characters. What Datta regards as the primary archesporial cell is really the megaspore-mother cell after the cutting off of the primary wall cell. The observations of Ghose and Alagh (1930) on *Cassia purpurea* agree with mine. They also noted hypodermal archesporium and the formation of the primary wall cell. This character, therefore, may be taken as characteristic of the genus. Paul (1937) has reported sub-hypodermal origin of the primary archesporium in *Tamarindus indica*. I consider his observations also doubtful.



Figs. 17–21. *Cassia glauca*.—Fig. 17. The primary archesporial cell. Fig. 18. The differentiation of the primary wall cell. Fig. 19. An ovule showing two megaspore-mother cells. Fig. 20. A linear tetrad of megaspores. Fig. 21. A mature embryo-sac. Fig. 19, × 800 ; the rest, × 1700.

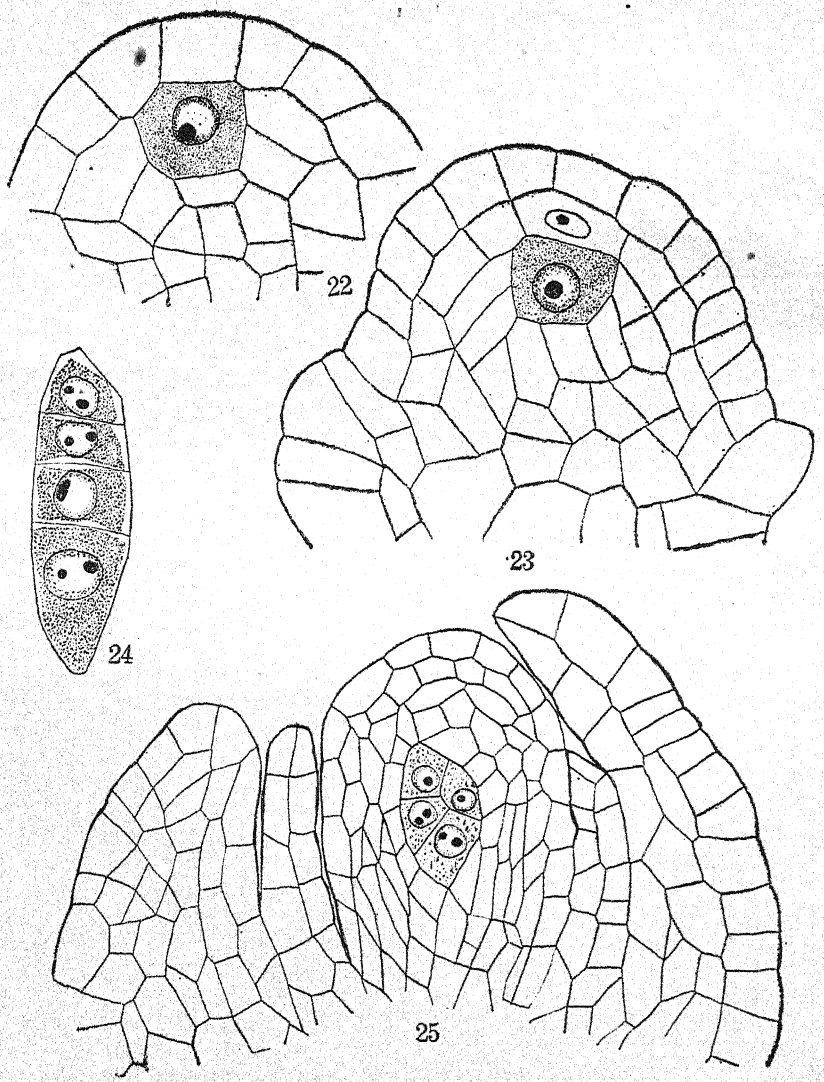
One functional archesporial cell and one megaspore-mother cell is the general character of the ovules of the different Cassias, but the occasional occurrence of two megaspore-mother cells has been observed in *Cassia glauca* (Fig. 19), *C. glauca* var. *suffruticosa* and *C. siamea*. Perhaps such exceptional cases are likely to occur in other species also

if a larger amount of material is examined. However, whenever two megaspore-mother cells were observed in an ovule, only one was seen to develop up to the tetrad stage. I did not come across any case of two tetrads or multiple embryo-sacs in an ovule. Occurrence of more than one megaspore-mother cells in an ovule has been previously noted by Datta (1935) in *Cassia tora*, and there are many similar instances reported among other Leguminosæ, *e.g.*, *Albizzia Lebbek* of the Mimosaceæ (Maheshwari, 1931), *Medicago sativa* (Reeves, 1930), *Melilotus alba* (Cooper, 1933), etc., belonging to the Papilionaceæ.

The primary parietal cell divides in all planes and by the time the two meiotic divisions in the megaspore-mother cell are completed, it gives rise to 4–5 layers of parietal cells (Figs. 9 and 31). Later the number of these layers increases to 8–10. Such extensive development of the parietal tissue seems to be characteristic of the Cæsalpiniaceæ and Mimosaceæ. In the Papilionaceæ, on the other hand, the parietal tissue is poorly developed. This agrees with the primitive character of the first two families and the more advanced position of the last family in the order.

The megaspore-mother cell after its differentiation undergoes a considerable period of rest and growth without any nuclear changes. It increases considerably both in length and breadth. The ovule also increases considerably during the megaspore-mother cell stage, so that the megaspore mother-cell becomes deep-seated. The meiotic divisions in all investigated species proceed in the normal manner. In *Cassia occidentalis* 14*n* chromosomes were counted during these divisions (Fig. 8 *a*). After the first meiotic division the mother cell is divided into two dyads by a transverse wall, which does not lie exactly in the middle (Figs. 8 *a*, *b* and *c*). The dyads are thus of unequal size, the chalazal one being larger. The second meiotic division in the two dyad cells generally does not proceed simultaneously. It starts earlier and proceeds more actively in the chalazal dyad than in the micropylar, so that in some cases even when the division has been completed in the chalazal dyad, the micropylar dyad is in the telophase stage (Fig. 8 *c*). Due to the difference in the size of the dyads, the megaspores formed from them also show slight size differences. The two chalazal megaspores are slightly larger than the two micropylar ones. The four megaspores are generally arranged in a linear order (Figs. 20, 24 and 31), but a T-shaped arrangement of the megaspores (Figs. 8 *c* and 9) was also seen in several instances in almost all species. In addition to this variation, in one ovule of *Cassia glauca* var. *suffruticosa* one megaspore-mother cell was observed to have given rise to an isobilateral tetrad of megaspores (Fig. 25). In this ovule there were two megaspores. One of these had formed this exceptional type of tetrad. The other was still in the megaspore-mother cell stage. It is not illustrated in the figure. Exceptional occurrence of isobilateral tetrads of megaspores in the flowering plants has been previously observed by Ducamp (1902) in *Fatsia japonica*, Greco (1930) in *Myrtus communis*, and Capoor (1937) in *Urginea indica*.

In all the species studied during the course of the present investigation the chalazal megaspore is found to develop into the embryo-sac (Figs. 14 and 31). The other megaspores degenerate, but the traces of the degenerating cells may be seen up to the 2-nucleate stage of the



Figs. 22–25. *Cassia glauca* var. *suffruticosa*.—Fig. 22. The primary archesporial cell. Fig. 23. Formation of the primary wall cell. Fig. 24. A linear tetrad of megaspores. Fig. 25. An ovule showing an isobilateral tetrad of megaspores. Fig. 25, × 900; the rest, × 1700.

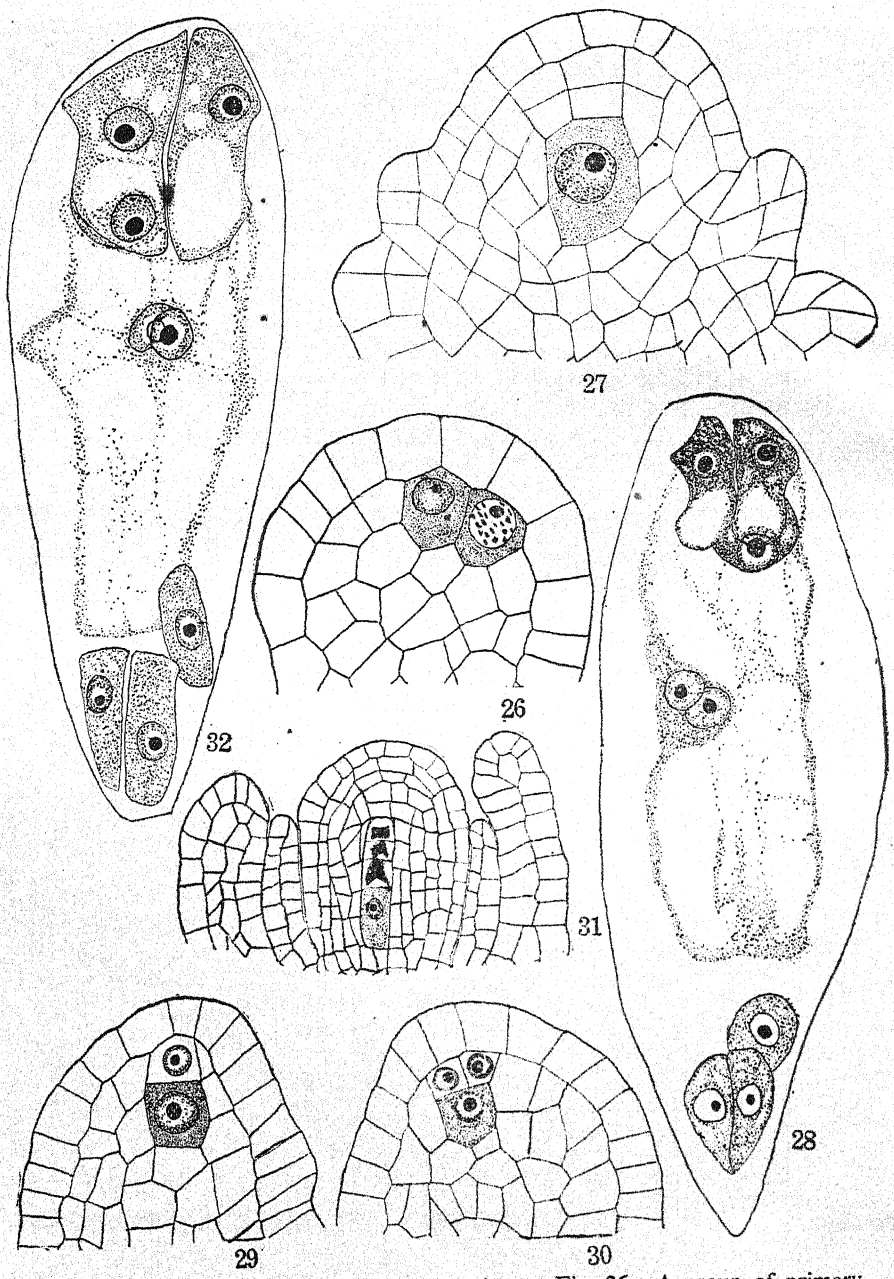
embryo-sac. Datta (1935) observed the same feature in *Cassia tora*, but Saxton (1907) and Ghose and Alagh (1933) found in *C. tomentosa* and *C. purpurea* respectively the second megaspore from the chalazal end developing into the embryo-sac. Such variation in the selection of the megaspores is common in the whole order Leguminosæ and was observed as early as 1881 by Guignard. From a study of about 40 species he concluded that in the Leguminosæ of the four megaspores of the tetrad either the innermost or the one next to it is the functional one.

The functional megaspore develops into the embryo-sac according to the *Normal*-type. It increases in size. Along with this vacuoles develop both above and below the central nucleus. The latter divides. The daughter nuclei move to the two poles of the embryo-sac and a central vacuole becomes prominent. The two nuclei at the poles undergo two more mitotic divisions, so that an 8-nucleate embryo-sac is formed with four nuclei at either end. Three nuclei at the micropylar end organise into the egg-apparatus, three at the chalazal end into antipodals and the two polar nuclei are left in the central cell (Figs. 10, 15, 21, 28 and 32).

Both the egg and the two synergidæ are nearly pyriform. The egg is slightly larger than the synergidæ. It shows a large vacuole towards the micropylar end, while the nucleus and the cytoplasm are pressed towards the chalazal end. The synergidæ show a large vacuole in the chalazal half, while the micropylar half is densely filled with cytoplasm. The nucleus is found embedded in the cytoplasm just above the vacuole. In all species the synergidæ show prominent hooks and a distinct "filiform apparatus" at the time of fertilisation (Figs. 11, 21, 28 and 32).

The antipodals form definite cells (Figs. 10, 11, 21, 28 and 32). Datta (1935) reports that in *Cassia tora* the antipodals are not organised into cells but remain as free nuclei. As I have observed antipodal cells in all the species investigated by me, his observations appear to me quite erroneous. Even in one of his own figures he has represented one of the antipodals as a cell with a cell-wall around it. In all the Leguminosæ investigated so far the organisation of antipodal cells has been noted. The antipodals in all Cassias are quite prominent and persist till the time of fertilisation. They often develop large vacuoles. In *Cassia glauca* var. *suffruticosa* they are sometimes even more prominent than the egg-apparatus. In *Cassia tomentosa*, Saxton (1907) mentions the presence of more than three antipodals, but I have not come across any such case in my material.

The two polar nuclei meet near the egg-apparatus or the middle of the embryo-sac. Here they remain together for a long time, but fuse only just before fertilisation. In *Cassia occidentalis*, the two polar nuclei just before fusion have been observed to enter the prophase stage and show the chromosomes quite distinctly (Fig. 11).



Figs. 26–32.—Figs. 26–28. *Cassia marginata*.—Fig. 26. A group of primary archesporial cells. Fig. 27. Apical region of an ovule with a megaspore-mother cell. Fig. 28. Mature embryo-sac. × 1700. Figs. 29–32. *Cassia siamea*.—Fig. 29. An ovule showing the differentiation of a primary wall cell and the megaspore-mother cell. Fig. 30. The same as Fig. 29 but showing the anticlinal division of the primary wall cell. Fig. 31. An ovule showing a linear tetrad of megaspores. Fig. 32. A mature embryo-sac. Fig. 31, × 900; the rest, × 1700.

SUMMARY

The development of the ovules and embryo-sac has been studied in *Cassia occidentalis* Linn., *C. obtusifolia* Linn., *C. glauca* Lamk., *C. glauca* Lamk. var. *suffruticosa* Koenig., *C. marginata* Roxb. and *C. siamea* Lamk. The ovules in all species are anatropous, with a slight tendency towards amphitropy, and bitegmic. The integument initials appear only after the primary archesporial cell has cut off the primary wall cell. The micropyle is somewhat zigzag and is formed largely by the outer integument. Further, in the region of the micropyle the outer integument for a short distance is separated from the inner by a small air-space. The nucellus is quite massive. The formation just before fertilisation of an epidermal cap at the micropylar end of the nucellus with a small beak projecting into the micropyle is characteristic. The epidermis of the funicle and the adjacent part of the outer integument on the outer side remains meristematic for a long time and close to the hilum grows out into a short hump-like structure, which persists throughout the life of the ovule.

The primary archesporium in all species is hypodermal and a primary wall cell is always formed. The earlier records about the occurrence of sub-hypodermal archesporium in some species of *Cassia* appear to be all doubtful. The megaspore-mother cell gives rise to a linear or T-shaped tetrad of megaspores, of which the chalazal develops into an 8-nucleate embryo-sac according to the normal type. In one instance in *C. glauca* var. *suffruticosa* an isobilateral tetrad of megaspores has been observed. The synergidæ are prominently hooked and show the filiform apparatus. The egg is pyriform and slightly larger than the synergidæ. The antipodals are definite cells and persist till the time of fertilisation. The two polar nuclei meet near the egg-apparatus. They fuse only just before fertilisation.

In conclusion I wish to express my sincere thanks to Dr. A. C. Joshi for his kind advice and help throughout the progress of the work.

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THE PLACE OF ANGIOSPERM EMBRYOLOGY IN RESEARCH AND TEACHING*

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In the history of Angiosperm Embryology there have been three distinct periods : the *first* in which the chief aim was to unravel the fundamental facts regarding the development of the pollen and embryo-sac, and the processes of fertilisation and seed formation ; the *second* in which interest centred largely round a study of comparative embryology and an evaluation of the data thus obtained for the improvement of the existing systems of classification ; and the *third* and most recent in which Embryology has become an experimental science like Physiology and Cytology, where one tries to study such problems as the storage of pollen and its germination, the receptivity of the stigma, fertilisation and fruit-setting, etc., and the optimum conditions required for them.

Descriptive Embryology

It is not necessary to spend much time on the first of these, *i.e.*, Descriptive Embryology, as most of the facts relating to the course of development of pollen, embryo-sac, endosperm and embryo had become clear towards the close of the last century through the efforts of Amici, Schleiden, Hofmeister, Strasburger, Treub, Guignard, Nawaschin and others, and are now a commonplace in all textbooks of botany. A very good summary of this work was given by Coulter and Chamberlain in the year 1903 and it was followed later by the publication of Schnarf's (1929) "Embryologie der Angiospermen", which is at present the most important and exhaustive treatise on this subject. Although little that is fundamentally new has probably been discovered since then, many errors and misinterpretations made by previous workers have been corrected and a mass of valuable information has been added regarding certain details concerned with the formation of the male gametes, the types of embryo-sac development, the cytology of fertilisation, the origin and function of endosperm haustoria and the development of the embryo. Work of this type is still in progress but the results will not be proportionate to the time spent unless a worker devotes his attention to just one aspect of the life-history in which he is most proficient and studies this in as many plants as possible. It is in this way that Finn in the Ukraine and Wulff in Germany and recently also some workers in the U.S.A. have been able to discover a number of important facts on the structure and development of the male gametes and Souèges in France on the development of the embryo in a large number of angiospermous families.

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* Presidential Address delivered before the 24th Annual Meeting of the Indian Botanical Society held at Nagpur, on January 3rd, 1945.

Phylogenetic Embryology or Embryology in relation to Systematic Botany

In the second period, which may be said to have commenced with the beginning of this century, embryology began to be used as an aid in the improvement of our systems of classification, the most important contributions in this line having come from Sweden (Stockholm, Uppsala and Lund), Germany (Bonn, Berlin and Vienna), and the U.S.A. (Chicago, Baltimore and California). A great impetus was given to such studies by the publication of Schnarf's excellent handbook entitled "Vergleichende Embryologie der Angiospermen" in which the author has summarised the existing state of our knowledge of the embryology of each family and added a number of valuable suggestions and comments at the end of each order. Most of the embryological work done in India has followed the publication of this book, to whose author we owe a debt of gratitude which cannot be expressed adequately in words. Although I alone among the Indian embryologists have had the privilege of working with Prof. Schnarf, yet all have gained considerable inspiration and insight into this difficult field through the medium of his publications.

As the value of embryology in questions relating to systematic botany does not appear to be sufficiently appreciated in this country by those who are engaged in other lines of study, it is necessary to consider this aspect in some detail.

It is a matter of common knowledge that on the basis of external morphology of the vegetative organs a genetical relationship may sometimes be inferred to exist between plants which belong to widely separated groups (*Equisetum* and *Casuarina*; *Ephedra* and certain Asclepiads; some cacti and Euphorbias). Taxonomists therefore take recourse to the flower as it is a more conservative organ than the stem and leaf. But, if we assume phyletic trends in the external morphology of the flower, why not in the internal structures, for these must be still more conservative (being less amenable to environmental influences) and therefore of special value in judging the proper position of certain doubtful groups? I am told that the zoologist would ordinarily refuse to assign an unknown animal to its systematic position until he has had an opportunity of examining its internal organs. No one can doubt that the same should be done with plants also, and if anything has prevented *us* from applying the anatomical and embryological method on a large scale, it is only the greater labour involved in it. The work *has* however to be undertaken now on a larger scale than ever as the systematist has taken us almost as far as he could towards our goal of a natural system of classification and can hardly make much headway without our help and co-operation.

Before proceeding further I must now enumerate such characters in the embryology of an angiosperm which are usually considered to be of major value in delimiting the larger plant groups :—

1. *Anther tapetum.*—Whether it is of the glandular or the amœboid type.

2. *Quadripartition of the microspore mother-cell.*—Whether it takes place by furrowing or by the formation of cell-plates.

3. *Development and organisation of the male gametophyte.*—Number and position of the germ pores and furrows ; adornments of the exine ; place of formation of the generative cell ; number and shape of the nuclei in the pollen grain at the time of its discharge from the anther.

4. *Development and structure of the ovule.*—Number of integuments and the alterations in structure which they undergo during the formation of the seed ; presence or absence of vascular bundles in the integuments ; shape of the micropyle, whether it is formed by the inner integument or the outer or both ; the presence or absence of an obturator.

5. *Form and extent of the nucellus.*—Whether it is broad and massive or thin and ephemeral; presence or absence of a hypostase; the place of origin of the integument or integuments, whether close to the apex of the nucellus (as in the Rubiaceæ) or near its base (as in the Orchidaceæ) ; persistence or disappearance of the nucellus in the seed.

6. *Origin and extent of the sporogenous tissue in the ovule.*—Nature of the archesporium, whether it is one-celled or many-celled ; presence or absence of wall layers ; the presence or absence of periclinal divisions in the cells of the nucellar epidermis.

7. *Megasporogenesis and development of the embryo-sac.—i.e.*, to which of the following main types or its modifications does it correspond : Normal, *Oenothera*, *Allium*, *Peperomia*, *Fritillaria*, *Adoxa*, *Plumbago*, *Plumbagella*, etc. ?

8. *Form and organisation of the mature embryo-sac.*—Shape of the embryo-sac and the number and distribution of its nuclei ; an early disappearance or otherwise of the synergids and antipodal cells ; increase in number of antipodal cells, if any ; formation of haustoria, if any, from some part of the embryo-sac.

9. *Fertilisation.*—The path of entry of the pollen tube ; the interval between pollination and fertilisation ; any tendency towards a branching of the pollen tubes during their course to the ovule.

10. *Endosperm.*—Whether it is of the nuclear, cellular or Helobiales type, and direction of laying down of the first wall in such cases where it is cellular ; presence or absence of endosperm haustoria and the manner in which they are formed if present ; nature of food reserves in endosperm cells.

11. *Embryo.*—Relation of the proembryonal cells to the body regions of the embryo ; form and organisation of the mature embryo ; presence or absence of suspensor haustoria.

12. *Certain abnormalities of development.*—Apomixis, polyembryony, parthenogenesis, etc.

While these are the most important characters usually taken into account in systematic studies, there are many others which it is

difficult to put down in writing. Indeed, as a very competent embryologist (Mauritzon, 1939) recently remarked, the resemblances and differences in the embryological characters of the members of a family are sometimes of such a fine type, that they can neither be brought out in words nor even in a drawing but can only be appreciated under the microscope. He nevertheless considers them to be of distinct value in delimiting the smaller groups and in determining their interrelationships with one another.

Let us now take some specific instances where embryology has rendered an important service in the determination of the proper position of some difficult families or in giving a new orientation to our ideas of their affinities.

The relationships of the family *Empetraceæ* exercised the minds of systematists for a long time and it has been placed by some authorities in the Monochlamydeæ, and by others in the Sapindales or the Celastrales. Samuelsson's work (1913) has definitely shown however that its proper place is with the Bicornes, a group which is characterised by the following well-marked embryological features :—

1. Absence of a fibrous layer in the anthers.
2. Presence of a glandular tapetum which does not become amœboid.
3. Pollen grains remaining together in tetrads.
4. Ovule with a single integument and a thin ephemeral nucellus which completely disappears in later stages so that the embryo-sac lies in direct contact with the integumentary tapetum.
5. Absence of parietal cells in the ovule, the hypodermal archesporial cell functioning directly as the megaspore mother-cell.
6. Embryo-sac of the monosporic eight-nucleate type with small ephemeral antipodals.
7. A hollow and fluted style which connects the lumen of the ovary with the outside and along which the pollen tubes make their way into the ovary.
8. Endosperm cellular, the first two divisions being transverse and giving rise to a row of four cells placed above one another.
9. The formation of endosperm haustoria at both ends of the embryo-sac, micropylar as well as chalazal.
10. A single-layered seed-coat formed from the outermost layer of the integument, the remaining layers becoming absorbed during the growth of the embryo-sac and embryo.

All these are perfectly standard stages in Erican embryology, a combination of which is not known to occur in any other order except the Bicornes. The Empetraceæ show a close correspondence in all respects, while the Sapindales and Celastrales differ from them (Bicornes) in so many ways that Samuelsson may be said to have established his point of view fully and completely. Hutchinson's assignment of the Empetraceæ to the Celastrales is therefore considered

by Schnarf (1933, p. 283) to be due to nothing but a "ganz besonderer Verständnislosigkeit".

On the other hand, the Lennoaceæ, which have sometimes been placed in the Bicornes (Hutchinson, 1926) certainly do not belong here. In the very first place, the equality in number of their stamens and corolla-lobes (contrasted with the obdiplostemony of the Ericales), the alternation of the parts, the adnation of the filaments to the corolla and the dehiscence of the anthers by longitudinal slits, form a weighty objection against this view. Add to these the fact that the Lennoaceæ have a short and solid style, a normally developed endothecium, pollen grains separate from each other, and a seed-coat which is more than one-layered. Svensson (1923) and Copeland (1935) therefore correctly consider the assignment of the Lennoaceæ to the Bicornes as quite untenable on embryological as well as other grounds and suggest that they might more reasonably be placed among the Tubiflorales as a separate suborder occupying a primitive position.

Let us now pass on to another group, the Cactaceæ. F. Vaupel (1925), in the latest edition of *Engler-Prantl's Pflanzenfamilien*, writes that there is hardly a family in the plant kingdom, the allocation of which has allowed so much scope to individual tastes as this. Wettstein placed it in the Centrospermales and Engler-Prantl in a separate order Opuntiales near the Passifloraceæ. Hutchinson has erected the order Cactales and placed it closest to the Cucurbitales.

The views of the great Viennese systematist have received very definite confirmation in this respect from the works of two embryologists, Mauritzon (1934) and Neumann (1935). Although additional work on this family would be welcome, the following features seem to be well established :—

1. A secretory tapetum of parietal origin.
2. Division of pollen mother-cells of simultaneous type.
3. Pollen grains tri-nucleate at the time of shedding.
4. Ovules campylotropous with strongly curved and massive nucelli.
5. Two integuments ; and the swollen lips of the inner, which alone forms the micropyle, protruding out to approach the funiculus.
6. A hypodermal archesporial cell which cuts off a wall cell.
7. A nucellar cap formed by periclinal divisions of the cells of the nucellar epidermis.
8. Embryo-sac of the Normal type.

Several other characters, for which a reference may be made to Frl. Neumann's original paper, point to the conclusion that the Cactaceæ belong to the Centrospermales and form a sort of bridge between the Aizoaceæ and Portulacaceæ. An interesting point, which has probably been overlooked by several workers but is nevertheless of considerable importance is the presence, in the chalazal part of the ovule, of a "Hohlraum" or "Luftspalt" between the two integuments and sometimes also between the inner integument and the nucellus.

This is quite distinctive of a number of other families belonging to the Centrospermales and its occurrence in the Cactaceæ is therefore of great significance.

On the other hand, a comparison of the embryology of the Cactaceæ with that of the Passifloraceæ offers so little by way of resemblance that any close relationship between them appears to be most unlikely.

Take again the Onagraceæ, in which the genus *Trapa* has long been considered to occupy a somewhat anomalous position. All the plants of this family so far investigated show a monosporic four-nucleate embryo-sac, *Trapa* alone being an exception with an eight-nucleate embryo-sac and a well-developed suspensor haustorium. From the embryologist's standpoint this strongly supports the case for a removal of this genus to a separate family—a course which has now been adopted by some systematists by erecting the family Hydrocaryaceæ for its reception. Regarding the relationships of the Onagraceæ with other families of the order Myrtales, it seems very likely that it has been derived from the Lythraceæ (Tischler, 1917) in which the ephemeral antipodals show the way to a complete omission of the chalazal part of the embryo-sac, leading to the four-nucleate condition of the Onagraceæ. I understand that this view is not challenged by systematists.

In Prof. Schnarf's laboratory at Vienna, some very important work has been done on the embryology of the Liliaceæ and Amaryllidaceæ, which has a great bearing on the interrelationships of the various sub-families and tribes included under these large and difficult families.

Taking the sub-family Lilioideæ, Engler (1888) divided it into the tribes Tulipeæ and Scilleæ. Schnarf (1929) stressed the sharp embryological differences between them, and in the second edition of the "Natürlichen Pflanzenfamilien" Engler and Prantl (1931) removed the Scilleæ from the association giving it the status of an independent sub-family, the Scilloideæ, so that the name Lilioideæ is now synonymous with the former Tulipeæ. Frl. Rosalie Wunderlich, a pupil of Prof. Schnarf, has again (1937) stressed the great contrast between the two (see taole below) and even hinted at the desirability of separating them into two distinct families. She rightly points out that in more than one respect the Lilioideæ appear to be a derived group while the Scilloideæ are more primitive ; the latter should therefore be placed *before* the former and not after as it has been done by Engler and Prantl.

Dr. Wunderlich further adds that the Scilloideæ themselves fall into two tribes : one with the Helobiales type of endosperm (*Ornithogalum*, *Muscari* and *Puschkinea*) which she calls the *Ornithogalum* group and the other with the *Nuclear* type including the genera *Hyacinthus*, *Scilla*, *Camassia* and *Galtonia*.

	Scilloideæ	*Lilioideæ*
1.	Parietal cell always present in ovule	Parietal cell absent
2.	Embryo-sac of *Normal* or sometimes *Allium* type	Embryo-sac of *Fritillaria* type

	Scilloideæ	*Lilioideæ*
3.	Endosperm of *Nuclear* or *Helobiales* type	Endosperm of *Nuclear* type
4.	Embryo large, occupying almost the entire length of the seed	Embryo small (*Tulipa, Lilium, Fritillaria, Erythronium*, etc.) and occupies only a small space in the seed
5.	Generative cell small and slender, not easily stainable with acetocarmine	Generative cell large and broadly spindle-shaped, staining easily with acetocarmine
6.	Male nuclei ± spherical	Male nuclei ± elongated
7.	Chromosome no. variable	Chromosome no. usually 12
8.	Raphides present	Raphides absent
9.	Septal nectaries present	Septal nectaries absent

The systematic position of the Moringaceæ has long been a matter of some doubt. The astonishing observations of Rutgers (1923) on the embryo-sac and embryo of *Moringa oleifera* only increased this element of uncertainty. He reported in this plant the absence of a parietal cell in the ovule and the presence of a five-nucleate embryo-sac and a free nuclear embryo. My pupil, Prof. V. Puri of Meerut (1940), has shown that a parietal cell is *present*, the embryo-sac is of the normal *eight-nucleate* type and what Rutgers considered to be a free nuclear embryo is merely a group of some *endosperm nuclei* at the micropylar end of the sac, the fertilised egg having escaped his notice altogether! The resemblances which the Moringaceæ show to the Capparidaceæ in embryology and carpel morphology make it seem fairly certain that their correct position is in the order Rhœadales and the place assigned to this family by Hutchinson—between the Capparidaceæ and Tovariaceæ—is therefore justified.

Although provisionally placed with the Rosales, there has always been some doubt regarding the interrelationships of the Podostomaceæ with the other families of this order. The extensive work on the embryology of the Crassulaceæ and Saxifragaceæ done a few years ago by Mauritzon (1933) has however brought out certain features which make it almost certain that the Podostomaceæ are much reduced apetalous derivatives of the Crassulaceæ. The peculiar structure of the ovules of the former appears to be brought about merely as the result of a continuation of the reduction already seen in the Crassulaceæ and *Crassula aquatica*, in particular, whose mode of life is somewhat similar to that of the Podostomaceæ and which has the most reduced endosperm in the Crassulaceæ, may well form a transitional stage leading to the complete suppression of this tissue in the Podostomaceæ. A striking agreement between the two families is the presence of a highly developed suspensor haustorium, a feature which in Mauritzon's (1939, p. 38) opinion offers "such an eloquent proof" of their relationship "as to convert many doubters".

It is possible to add numerous other instances where embryology has rendered signal service to systematic botany, but considerations of time and space forbid me from citing them here. I believe that a stage has now arrived when we should try to have an embryological formula for each family as a supplement to the well-known floral formula so commonly used by systematists. To make my meaning clear I give below the embryological formula of the family Alismaceæ with which I have been particularly familiar as the result of the work done on it by my pupil Dr. B. M. Johri of Agra and later by Balwant Singh and myself (Maheshwari and Singh, 1943) at Dacca :—

ANTH.-TAP. (amœboid) ; DIV. OF P.M.C. (succ.) ; P. (3-nucl.) ; OVULE (2-integ. ; anat.) ; PAR. CELL (absent) ; E.S. (*Allium* T.) ; END. (*He.* or *Nu.* T.) ; EMB. (*Sag.* T.).

Put in plain English this means that the anther tapetum is of the amœboid type ; the divisions of the pollen mother-cells are successive ; the pollen grains are 3-nucleate at the time of shedding ; the ovule is anatropous and has two integuments ; no wall cell is cut off by the primary archesporial cell which functions directly as the megaspore mother-cell ; the embryo-sac development is of the *Allium* type ; the endosperm is of the *Nuclear* or *Helobiales* type ; and the embryo is of the *Sagittaria* type.

It may be possible by means of further abbreviations to include this information in a still shorter space and to make other improvements so as to devise a symbolisation which will be internationally acceptable. I suggest that this point may be discussed, by those who are interested, in the pages of one of our monthly journals like *Current Science.*

The embryologist would however be glad to admit that he lays no claim to erect a phylogenetic scheme of his own. Indeed there are some very definite limitations to the embryological method, for, owing to parallel, convergent and regressive evolution, similar embryological characters may often be found in widely separated groups and if a system of classification were to be set up on such considerations alone, some rather fantastic results are bound to ensue. But, with the main lines of phylogenetic classification already chalked out by the systematist, it is possible for the embryologist, the cytologist and the anatomist to use this as a background and to help him in making it more perfect. A natural system has to be discovered (for it is already there) and not invented. In order to do this we have to do real detective work and take the aid of every branch of botany. Once a group has been assigned to its true place, every character that is studied will only serve to strengthen its position. On the other hand, if there are any discrepancies, they will be brought out in a more glaring fashion by the study of its internal structures (as these are less influenced by the environment) than the external.

I understand that in some of the big herbaria of the U.S.A. steps are being taken to have along with the dried specimen a preparation or two showing the structural features of its wood. This is a laudable effort, but it ought to be extended still further so as to include

in each case about half a dozen preparations of the pollen, ovule and seed as well.

Applied and Experimental Embryology

Now we come to Applied Embryology which has evolved, for the most part, only during the last two decades or so. It is hardly possible to do any justice here to this subject, for although still in its infancy, it already has such a voluminous literature as to defy any attempt to review it in a few pages. I shall therefore satisfy myself merely by indicating some of the main lines on which work is being carried on in this field.

Before any improvement of our crop plants can be undertaken through breeding methods, it is necessary to have *in each case* a thorough understanding of the behaviour of the flower throughout its development and the setting of the fruit. Of the greatest importance in this connection are :—a study of the viability of the pollen and the optimum conditions for its storage and germination ; the receptivity of the stigma ; the rate of pollen tube growth under different conditions of temperature and humidity ; the interval between pollination and fertilisation and how it can be influenced by external conditions ; and the quantity of pollen necessary for a proper fruit set. Considerable work of this nature is being done in America and Russia, regarding the immense value of which in our breeding programmes there can be no question.

It is said that the Arabs put aside the pollen of the date palm from year to year so as to ensure a supply of dates even in the possible event of the male flowers failing to develop or the female flowers developing precociously. If we succeed in devising suitable conditions of temperature and humidity for storing the pollen of other cultivated plants, which normally is not so long-lived, we may be able to cross two varieties which flower on widely different dates or which are separated by considerable distances from each other. In the latter case it may be possible to transport the pollen by air from one place to another. We may hopefully envisage the possibility of opening one or more "pollen banks" in each country, where pollen of almost every important plant of economic value will be stored under optimum conditions and supplied to recognised workers, gratis or on a moderate charge.

Sterility and unfruitfulness are often caused by a very slow growth of the pollen tube. The flowers do not remain attached to the plant for an indefinite period and unless fertilisation takes place within a reasonable time, varying with the species under consideration, abscission takes place at the base of the style and fruit setting is consequently prevented. Premature as well as delayed pollination have the same result and we therefore need full information regarding all of our fruit trees and crop plants on the rate of pollen tube growth and the time when the stigma is most receptive. The optimum conditions for the germination of pollen also need to be investigated more fully. Tischler's (1910) discovery that much of the pollen of certain species of *Cassia* occurring at Buitenzorg fails to develop without an

outside supply of diastase illustrates the need of such work from various points of view.

Another aspect of applied embryology is a study of the possibility of obtaining a fruit set without the generally associated seed formation. There are a number of our edible fruits where the pericarp is the chief edible portion and the presence of seeds is neither necessary nor desirable. It was found possible in several cases to do away with the fertilisation of the egg cell and give the ovary the necessary stimulus for further development by the application of pollen extracts. This led to a chemical analysis of the latter which in turn opened the way for the induction of artificial parthenocarpy through the use of growth hormones (for literature see Maheshwari, 1940; Gustafson, 1942).

A very important paper was published in 1928 by C. A. Jørgensen, which showed the way to the induction of parthenogenesis in flowering plants. He pollinated the stigmas of *Solanum nigrum* with pollen from some other species of this genus. Most of the fruits were seedless but a few were found to have formed 2 to 8 seeds which gave rise to haploid plants of *Solanum nigrum*. An embryological study showed that in certain cases the foreign pollen had germinated successfully and the pollen tubes had also reached the embryo-sacs, but the male nucleus which enters the egg cell eventually disintegrates and disappears. The egg cell thus develops by itself into the embryo, stimulated no doubt by the entry of the male nucleus. The plants produced from the resulting seeds are therefore haploids resembling the maternal parent. In other cases, apparently, the male gamete alone may give rise to the embryo as inferred from the characters of the offspring which resembles the paternal parent but as far as I am aware the cytological and embryological processes leading to this condition are still unknown. Jørgensen's work opened the way towards the artificial production of haploids in a number of species and races. Although weak and valueless in themselves, they are of great utility in giving us an insight into the genetic constitution of the parent variety and for the production of homozygous diploids by a subsequent doubling of the chromosomes.

The effects of X-rays, colchicine treatment and exposure to extreme temperatures on the normal course of development are other aspects of recent cytoembryological research, which it is impossible to deal with here. It need only be said that while a fair amount of work has been done on the manner in which they influence ordinary mitotic and meiotic divisions, we are still very much in the dark about their effect on megasporogenesis, fertilisation and the development of other ovular structures like the endosperm and the embryo.*

The Place of Embryology in Botanical Teaching

At the end of this very short and imperfect sketch of the aims and scope of embryological research we may now consider another aspect of the subject, *e.g.*, its place in botanical teaching. That a study of embryology demands a more thorough training in microtechnique

* The important work, which is being done by Blakeslee and others on the artificial culture of excised embryos, will be reviewed elsewhere.

than is usually needed for other branches of plant science is a fact well known to everybody and it is perhaps for this reason that while much time is spent in our class-rooms on vegetative anatomy, in several universities little or nothing is shown to the students concerning angiosperm life-histories. A great opportunity is lost thereby of training them in the art of observation, reconstruction and critical interpretation. While granting that it is the advent of the microtome, as an instrument of precision in making serial sections, that has done so much for the recent progress in this science, it is not to be imagined that an elaborate scheme of fixing, dehydration, infiltration, imbedding, cutting, and staining is necessary in all cases. Much can be seen and shown by simpler methods. All stages of microsporogenesis and the maturation of pollen grains can be observed by making smears of suitable materials like *Tradescantia, Gloriosa*, etc., stained with either gentian violet or Feulgen (for technique see Darlington and La Cour, 1942). It is possible to mount the pollen grains of *Ottelia* and *Hydrilla* in acetocarmine and make a preparation, showing the vegetative and generative (or sperm) cells, even under the low power, in less than 5 minutes. Anthers of even herbarium specimens are often quite usable for such purposes (see Leitner, 1938). Some years ago Dr. Wulff and I (Maheshwari and Wulff, 1937) gave a schedule for making permanent mounts of pollen tubes to show the division of the generative cell and the organisation of the male cells. The common garden species of *Impatiens* is very good for this purpose as the pollen grains germinate readily and show the desired stages in only half an hour's time. Some of the Pontederiaceæ like *Monochoria* and perhaps several other plants may be equally suitable for the purpose. With the use of vital stains such slide cultures of pollen tubes may be used for studying the movement of the cytoplasm and the male gametes.

An observation of the stages in megasporogenesis and embryo-sac formation involves greater difficulties as the cells concerned are encased in several layers of other cells belonging to the nucellus and integuments, but Hillary (1940) has recently developed a technique by which he has been able to follow the development of the embryo-sac of *Lilium longiflorum* right from the megaspore mother cell up to the time of fertilisation and beyond, without cutting any sections. The ovules are here taken out from the ovary and the tissue around them removed as far as possible. Then they are fixed, washed, and stained with Feulgen's reagent in small vials or tubes. From the SO_2 water they are transferred to a drop of acetic acid placed on a slide and crushed under a coverslip. The author presents photomicrographs made from such preparations, which show the nuclei and chromosomes standing out quite distinctly in the colourless cytoplasm of the embryo-sac.

In his work on *Notonia grandiflora*, Ganesan (1939) used a somewhat similar method in order to select material of the right age for a study of the reduction divisions in the ovule. In this case an ovule is dissected out and mounted in a drop of acetocarmine mixed with an equal quantity of 1% safranin in 50% alcohol. By gentle and gradual increase of pressure on the coverglass, the nucellus is now freed from the

thick integument and in about half an hour's time the megaspore mother-cell nucleus is adequately stained for the purpose. By this method the author was able to exercise some judgment at the time of fixing the material and save much labour which would otherwise have been wasted if the cutting had been done at random. Poddubnaja-Arnoldi's (1938) "rapid method of embryological investigations" is essentially similar except that she recommends a mixture of acetocarmine and glycerine. She was thus able to follow the development in some plants upto the first stages in the formation of the embryo.

Suitable material for watching the process of fertilisation without recourse to section cutting has not yet been found* but one may try for this purpose such plants as *Torenia* and *Utricularia* in which the nucellus degenerates early and the upper part of the embryo-sac protrudes out of the micropyle so that it is naked and therefore more readily observable. The styles and stigmas of *Portulaca, Ottelia* or *Monochoria* should also be examined in order to follow the course of the pollen tube from the stigma to the ovule. A treatment with lactophenol and cotton blue often facilitates such observation.

That certain interesting features of endosperm morphology can be brought out more clearly from whole mounts of suitably dissected material than from sections, is shown by the work of Kausik (1939) on *Grevillea*. Dr. Kausik discovered in this plant a curious "worm-like" structure, which he calls the "vermiform appendage", formed by the chalazal part of the endosperm. This was missed by earlier observers since they used only sections which naturally fail to give any complete or intelligible picture of this tortuous organ.

There is perhaps no way of studying the development of the embryo except by cutting thin sections of the ovule but favourable material may yet be discovered in which the technique so successfully used by Buchholz (1938) in the study of conifer embryogeny will be found adaptable for at least some stages of this process. Also, in certain cases the seed coat may be so transparent (B. G. L. Swamy tells me that this is the case in many orchids) that it is possible to see the embryo in whole mounts of the seed without recourse to sectioning.

Let me explain why I am so keen that students should cut sections and make whole mounts or try other methods so as to see the entire process of development of the gametophytes and embryo in angiosperms as an essential part of any course in botany. This is because there are few other spheres of botanical study which offer a similar variety of technical problems or opportunities for the development of a critical attitude which is the most important quality that a young worker must learn to imbibe. I hope to be excused for citing here the case of a student who, having just taken the Master's degree, placed before me with great satisfaction a set of slides in which he claimed to have seen "all" stages of the development of the embryo-sac—1-nucleate, 2-nucleate, 3-nucleate, 4-nucleate, 5-nucleate, 6-nucleate,

* *Monotropa*, which is said to be very favourable for this purpose, is unfortunately not available in this country except in the hills and other inaccessible places, and the plant is not amenable to cultivation owing to its saprophytic habit.

7-nucleate and 8-nucleate. When the preparations were scrutinised it was found that all the sections were of mature embryo-sacs, but as the nuclei were spread apart into several sections, they were counted as they came, some here and others there, and imagined to be *stages in development* of an embryo-sac. Again, scores of students get away through a university course with the impression, gained from a study of book figures, that the integuments are lateral processes developing from the right and left sides of the nucellus, although a cross-section of the ovule or a whole mount of the same would have easily convinced them that it is not so.

I should add that it is not merely the student who makes mistakes but that even the experienced researcher is liable to be misled into such erroneous interpretations as may in some cases ruin his reputation as a scientific worker. Indeed, there are so many pitfalls in the correct interpretation of the material that the embryologist must always remain as watchful and alert as the worker on fossils. As an instance may first be cited the case of the *Lilium* embryo-sac whose development was repeatedly and very intensively studied by the most competent workers like Strasburger, Coulter, Mottier, Guignard and others. And yet, all of them were mistaken as the excellent work of Bambacioni (1928) showed a few years later. The embryo-sac of *Plumbagella*, long considered to be the most reduced among angiosperms, has turned out to be but a modification of the type seen in *Fritillaria* and *Lilium* (Fagerlind, 1938 ; Boyes, 1939). *Plumbago* has also been shown to have a development very different from that originally described for it by Dahlgren (see Haupt, 1934) and this has now been confirmed for another member of the same family, *Vogelia indica*, found in Rajputana (Mathur and Khan, 1941). In *Euphorbia heterophylla*, Sanchez (1938) recently reported a tetrasporic embryo-sac which has on reinvestigation turned out to be of the normal monosporic type (Maheshwari, 1942). Then again, the embryo-sac of *Rudbeckia*, which Palm (1934) believed to be of an entirely new type has been found to correspond with the now well-known *Fritillaria* type (Maheshwari and Srinivasan, 1944).

Another kind of error which is of frequent occurrence is the mistaking of the integument for the nucellus or *vice versa*. Even so recently as 1938 Houk fell into such an error in the case of the ovule of *Coffea* and in his confusion stated that the tissue may be regarded as an "integument-nucellus". Joshi (1938), Mendes (1941) and several other workers have shown that the nucellus and integument are both formed normally but the former soon disappears as is usual in most Sympetalæ. A similar mistake appears to have been made by Pannochia-Laj (1938) who writes that in *Lochnera rosea* the ovule is peculiar in that it is not possible here to delimit the nucellus from the integument. In another genus, *Fouquieria*, the structure which was supposed to be a " massive " nucellus is really the inner integument, the former being extremely reduced and ephemeral (see Khan, 1943). Evidently Dr. Woodcock (1943) has also been misled when he says that in *Ipomæa rubro-cærulea* the ovule "has no distinct integument" and the micropyle is formed by an "invagination at the end of the ovule next to the funiculus (see Maheshwari, 1944*b*).

The origin of the haustorial processes in the ovule has been another fruitful source of errors and misinterpretations. To mention only two such cases, Heinricher (1931–32), in his monograph on the genus *Lathræa*, stated that the micropylar haustoria are formed from the synergids and the chalazal from the antipodal cells. This was promptly contradicted and disproved by Glišic' (1932) who made a thorough study of *Lathræa squamaria* and found that both the haustoria are formed from the endosperm. A similar mistake made by G. O. Cooper (1942), working on *Lobelia cardinalis*, has already been commented on by me a few months ago (Maheshwari, 1944*a*).

Without citing further instances, I shall conclude this portion of my address merely by saying that even if the laboratory work in this subject makes greater demands upon the energy and resourcefulness of the teacher, this should not be grudged, as through this the young pupil gets such a stimulus for his mental development as is sure to be of use to him ever afterwards in his future career.

The Future

And now we proceed to the future.

It is said by some that the days of descriptive embryology are now over. This is far from true in my opinion. We need more of such investigations and will continue to do so for a long time. What needs to be emphasized, however, is that the descriptions must be full and accurate and the interpretations checked as critically as possible with preparations of the highest quality. As I have mentioned earlier, results of greater value may be expected if attention is focussed on a comparative study of only one aspect of the life-history at a time, *viz.*, male gametophyte, ovule, embryo-sac, pollen tube, etc. Each of these requires the study of a vast amount of literature and sometimes a technique different from that used for the rest.

With respect to phylogenetic embryology there is a great scope in our country, for we have representatives of a number of families in India, Burma and Ceylon, which have either received little or no attention or which deserve more intensive study than has so far been bestowed upon them. I mention below the names of a few but the list is by no means exhaustive and is capable of amplification :—

Aristolochiaceæ
Balanophoraceæ
Berberidaceæ
Burseraceæ
Callitrichaceæ
Ceratophyllaceæ
Cornaceæ
Crypteroniaceæ
Dilleniaceæ
Dipterocarpaceæ
Droseraceæ
Ebenaceæ
Flacourtiaceæ
Fumariaceæ
Hippocrateaceæ
Loranthaceæ
Magnoliaceæ
Myristicaceæ
Myrsinaceæ
Nepenthaceæ
Nymphæaceæ
Pittosporaceæ
Podostemaceæ
Salvadoraceæ
Simarubaceæ
Sterculiaceæ
Symplocaceæ
Thymelæaceæ
Zygophyllaceæ
Cyperaceæ
Dioscoreaceæ
Eriocaulaceæ
Flagellariaceæ
Hæmodoraceæ
Juncaceæ
Lemnaceæ
Marantaceæ
Najadaceæ
Palmaceæ
Pandanaceæ
Stemonaceæ
Triuridaceæ
Xyridaceæ
Zingiberaceæ

A thorough investigation of so many families requires much time and patience and the participation of a band of workers properly trained in embryological methods. Fortunately we have a number of qualified embryologists at Mysore, Bangalore, Benares, Annamalainagar, Poona, Madras, Agra, Meerut, Calcutta and other places, and I venture to hope that, with the co-operation of some of our colleagues in Europe and America, we may be able to prepare in this country a new "Comparative Embryology of Angiosperms" written more or less on the lines of Schnarf's great work (now 15 years old) in which each worker will write an exhaustive and critical account of the embryology of the particular group with which he is most familiar as the result of his *own* researches, for the literature on the subject is now too vast to be surveyed in a satisfactory manner by any one person. Students of wood structure like Record, Bailey and Wetmore, of chromosomology like Tischler, and floral anatomy like Eames and Arber are working towards the same end for their particular subjects. I expect that some of us present here will live to see the day when few families of flowering plants will need to be assigned by guess work, and in any case, whether the end comes sooner or later, we must travel hopefully towards it.

One fact to be noted in this connection is that although the embryologist cuts sections of the flower at various stages of development, he frequently confines his attention to the development and organisation of the embryo-sac and the subsequent changes which take place *inside* it or to a study of the meiotic divisions in the pollen mother-cells, while the structure of the anther and ovary wall, the placentation, the integuments, the nucellus, and the chalaza are either dealt with in a more or less cursory fashion or not described at all. This is unfortunate as all of these yield characters of great systematic value. The structure of the seed and the fruit receives even less attention probably because of the difficulty of sectioning them, but a judicious use of dilute hydrofluoric acid can sufficiently soften them in many cases without causing any appreciable harm to the tissues.

Last of all we come to the comparatively new science of experimental embryology. This is a more difficult field, but one which is full of promise in so many ways. As some one once said, the plant breeder puts pollen on the stigma and 'prays' for results in the ovary! For a scientific explanation of his successes and failures and for finding out the ways and means of increasing the former and remedying the latter, he must turn to the cytologist and the embryologist. The work that has been started in recent years on the effect of X-rays, heat, chemicals, etc., on the artificial induction of mutations is still in its infancy and it opens up vast possibilities before us. Here the breeder, the cytologist, the embryologist and the physiologist, must all join hands so that not only do we get the maximum results from what we have but we may evolve new and still better varieties of plants and thus add to the health and happiness of the world.

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CHROMOSOMES OF *ERYTHRINA INDICA* LAMK.

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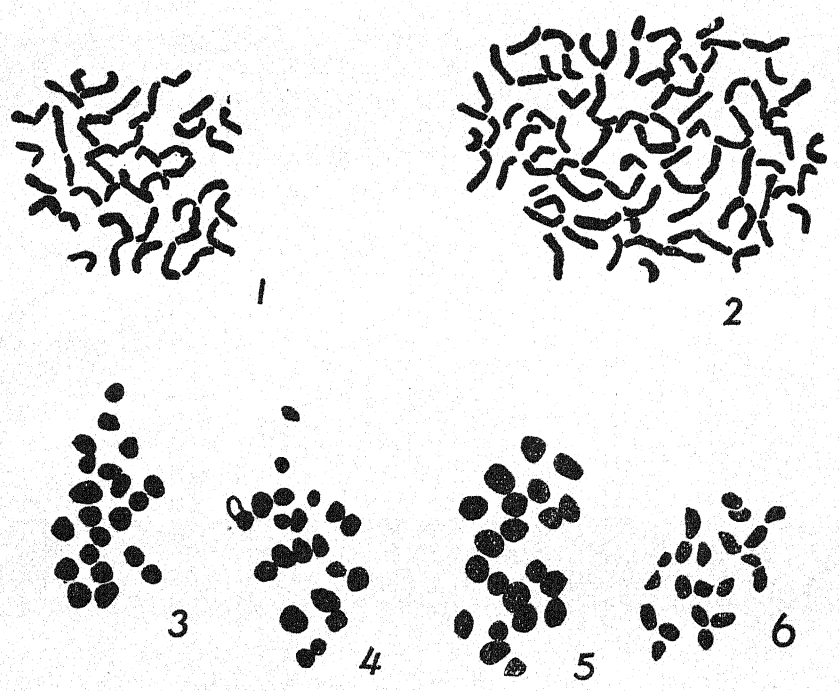
Received for publication on December 15, 1944

THE first observations on the chromosome numbers in the genus *Erythrina* L. (Fam. Papilionaceæ) were made by Tschechow and Kartaschowa, who reported for *Erythrina crista galli* L. [*Micropteryx crista galli* (L.) Walp.] in the same year in one of their papers (Tschechow and Kartaschowa, 1932*a*) *ca.* 40 and in another paper (Tschechow and Kartaschowa, 1932*b*) *ca.* 44 somatic chromosomes. The smallness of the chromosomes and their fairly large number in the root-tip cells might be the probable reasons for this obvious discrepancy. Next, Senn (1938) reported $2n = 42$ and $n = 21$ chromosomes in *Erythrina herbacea* L., and added that he too could not determine exactly the chromosome number in *E. crista galli*. The purpose of the present note is to report the chromosome number in the Indian Coral Tree, *Erythrina indica* Lamk., which grows wild along the Indian sea-coast and is widely planted in the gardens throughout the country for its large brilliant scarlet flowers. The materials for investigation, seeds and flowers, were obtained from trees growing at Guntur in the Province of Madras.

SOMATIC CHROMOSOMES

The somatic chromosomes were studied in root-tips obtained from germinating seeds. Most of the dividing nuclei showed 42 chromosomes (Fig. 1). These do not exhibit a wide range in size, but are small, slender objects, of nearly the same size and show either median or submedian attachment constriction. The somatic karyotype of *Erythrina indica* Lamk. thus appears to be identical with that of *E. herbacea* L. as sketched by Senn (1938).

During the examination of the sections of the root-tips, besides the monosomatic cells, some disomatic cells were also observed. The dividing nuclei of such cells showed 84 chromosomes (Fig. 2). Such cases of somatic doubling of chromosomes have been reported already in many Leguminosæ. Senn (1938) in his extensive work on the cytology of this family found tetraploid cells in *Albizzia Julibrissin* and *Cassia nictitans*, and Iyengar (1938) in *Cicer arietinum*, but the most comprehensive observations in this respect have been made by Wipf (1939) and Wipf and Cooper (1938 and 1940). They have reported the general occurrence of cells with tetraploid nuclei in the roots of several Leguminosæ, such as *Pisum sativum*, *Lathyrus latifolius*, *L. odoratus*, *Lespedeza tomentosa* and *Vicia villosa*, and find a definite



Figs. 1–6. *Erythrina indica* Lamk.—Fig. 1, Somatic metaphase showing 42 chromosomes. Fig. 2. Same showing a tetraploid nucleus with 84 chromosomes. Figs. 3–5. Polar views of Metaphase I ($n = 21$). Fig. 6. Metaphase II; only one plate of a P.M.C. is shown; $n = 21$. ×3,000.

relationship between the normal occurrence of disomatic cells in the roots of these plants and the formation of root nodules. The genetic significance of somatic doubling of chromosomes in restoring the fertility of sterile hybrids and in the origin of new species is already well known and need not be mentioned again here.

Meiosis

Observations on pollen mother cells undergoing meiosis showed $n = 21$ both at the I and II metaphase (Figs. 3–6). There are slight differences in size among the various bivalents at the I metaphase, but much importance need not be attached to this, as the size of bivalents in the polar views is determined by the presence and number of chiasmata (*cf.* Upcott, 1936). Polar views of meiotic chromosomes in *Erythrina indica* are also characterised by a marked degree of secondary association (Figs. 3 and 4). Groups of 2, 3 and 4 bivalents are quite common during the I metaphase and secondary association persists even in II metaphase.

Discussion

The following table summarises the chromosome numbers reported so far in the genus *Erythrina* L.:—

Chromosome Numbers in Erythrina *L.*

Species	n	$2n$	Author
E. crista galli L.		*ca.* 40	Tschechow and Kartaschowa (1932*a*)
Do.		*ca.* 44	Do. (1932*b*)
E. herbacea L.	21	42	Senn (1938)
E. indica Lamk.	21	42	This paper

The occurrence of $n = 21$ and $2n = 42$ chromosomes both in *E. herbacea* and *E. indica* make it very probable that in *E. crista galli* also there are 42 somatic chromosomes.

The occurrence of a rather high chromosome number in species of *Erythrina* as compared with most of the Papilionaceæ and secondary association both during the I and II meiotic divisions suggests that the genus *Erythrina* is of a polyploid nature. Taking into account that $n = 21$ is an unusual chromosome number in the Papilionaceæ, Senn (1938) remarked that this may indicate an ancestry through a 7 series or be the result of hybridisation from $n = 10$ and $n = 11$ ancestors with subsequent amphidiploidy. The latter suggestion, according to him, appears possible in view of the taxonomic position of the genus between forms with a basic number 10 and forms with a basic number 11. Future investigators of the cytology of *Erythrina* have to study this problem.

In the end, the author desires to express his appreciation to Dr. A. C. Joshi for his kind interest in the investigation and help in the preparation of this note.

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302-46 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent